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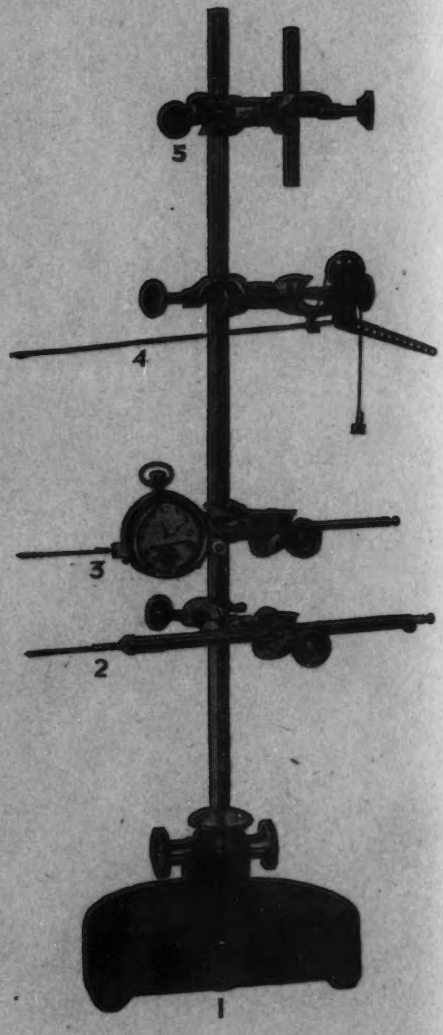

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IS A-VITAMIN SECRETED THROUGH THE SKIN?

Experimental Investigations on A-Vitamin in Wool Fat

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It is a well-known fact that a considerable change in the appearance of the fur is one of the first symptoms of avitaminosis in rats. The hair begins to bristle, becomes looser and even falls out. This is particularly the case around the eyes, when xerophthalmia is about to set in. Frequently a completely hairless zone is formed around the eye. At the muzzle and on the forehead the hairs often lie in a direction opposite to the usual one and give the animal a peculiar look, resembling that of a hedgehog. Apparently something must have happened to the skin itself, be it purely functional disturbances, pathological-anatomical changes, or both. It might be supposed that perhaps the skin has some special relation to the A-vitamin, and that this question might be worth experimental investigation. Accordingly the following experiments were made to ascertain whether A-vitamin is to be found in the secretions of the skin.

It is very difficult to get hold of skin secretions as these appear in very small quantities, while in animal-feeding experiments considerable quantities of the various substances are required if the results are to be reliable. Therefore wool fat from sheep was used as a source of material.

The official preparation, adeps lanae, was not used, as it was assumed that, even if A-vitamin were in the wool originally, it might have been destroyed in processing.

On this account the fat was extracted from the fresh, unwashed wool taken directly from the sheep, without the latter having been previously washed. As is well known, considerable quantities of fat are found in sheep's wool. Herbig (1894) found in raw wool from South America 13.2 per cent. But the fat content of wool is very different according to the climate and the breed of the sheep, and perhaps according to whether the sheep are fat or lean. The fat often sticks like yellow, small waxlike

lumps between the hairs, spread all over the whole wool, but mostly in the parts near the skin. Two kinds of wool fat have been examined, the one from Argentine sheep's wool and the other from Icelandic sheep's wool.

THE ARGENTINE WOOL FAT. Argentine sheep's wool was employed which was bought at a store in Copenhagen. It is not known whether the sheep were washed before the wool was shorn off, but it is stated that the wool was not washed and that it had been in the store for a couple of years. It was white, dirty and felt greasy. It was extracted with ether in a Soxhlet extraction apparatus and the greater part of the ether distilled off in the same apparatus. The extract then was quite yellow and viscid. The last traces of ether were removed in vacuo at a temperature of 50°C. for half an hour. The yellowish brown mass of fat which remained was used in the experiments. This mass is somewhat hard with a shining surface. It smells strongly of sheep's wool (a peculiar creosot-like smell)

TABLE 1

WOOL-FAT	BUTTER-FAT	PETRO-LEUM-ETHER	REACTION
cc.	cc.	cc.	
2.5	0	2.5	The liquid immediately becomes dark brown. No color reaction
1.0	0	3.0	The liquid immediately becomes dark brown. No color reaction
1.0	1.0	2.0	The liquid assumes a yellowish brown color, no color reaction
0.25	0.75	2.0	The liquid a little less brown but no color reaction
0	1.25	3.75	Typical reddish purple reaction which soon disappears

and tastes bitter. It melts at about 40°C., has an acidity value of 19.8, and an iodine value (Wijs' method) of 38.156.

Drummond and Watson (1922), Poulsson and Weidmann (1923), and N. Sjörslev (1924) have suggested a relation between the sulphuric acid reaction and the contents of A-vitamin in fat substances. As a supplement to the experiments on rats this reaction on wool fat was tried. Investigation was also made as to whether wool fat possibly possesses the capacity of destroying the sulphuric acid reaction in butter fat previously freed of casein, salts and water. As shown by Sjörslev, this is the case with heated pork fat. The method given by Sjörslev was employed; the wool fat is dissolved in petroleum ether and a few drops of concentrated sulphuric acid are added. The wool fat and butter fat are first melted together. The butter fat is first examined and gives the characteristic reddish purple sulphuric acid reaction.

This sample of wool fat shows results similar to those of Sjörslev with

heated pork fat which Fridericia (1923) has shown not only to be free from vitamin but besides to possess a vitamin-destroying capacity.

THE ICELANDISH WOOL FAT. The sheep on Iceland belong to a cross between several different breeds. They have long and coarse hair which is not so curly as, for example, that of merino sheep.

During summer most sheep go up into the mountains onto the rich pastures and live exclusively on the hardy and strong green mountain plants. In autumn the sheep have accumulated much fat on which they can live during the long winter with its short days. The wool used for the experiments was taken from sheep's skins immediately after these autumn-fat mountain sheep were slaughtered. It was not washed or treated in any other way. It was about 3 weeks old, white, only a little dirty and did not feel as greasy as the Argentine wool.

TABLE 2

WOOL-FAT	BUTTER-FAT	PETRO-LEUM-ETHER	REACTION
cc.	*cc.*	*cc.*	
2.5	0	2.5	The liquid immediately assumes a green color, but then changes to greenish brown, no reddish purple color
1.0	0	3.0	The liquid immediately assumes a green color, but then changes to greenish brown, no reddish purple color
0.1	0	3.0	Intense light green color which soon changes to yellowish brown
0	1.25	3.75	Typical reddish purple reaction which quickly disappears
1.0	1.0	2.0	Deep green color, soon changes to greenish brown
0.1	0.9	3.0	Reddish purple tint which is soon covered by a weaker but distinct green color

The wool was extracted, as described above, in a Soxhlet extraction apparatus. Most of the ether was then distilled off in the apparatus, the remainder being removed in vacuo at about 50°C. for half an hour. The fat thus obtained is light yellow, with a dull surface, half soft and melts at about 30°C. It smells strongly of sheep's wool and tastes bitter.

It has an acidity value of 6.39, an iodine value (Wijs) of 25.7514.

The sulphuric acid reaction was determined as before, the result being shown in table 2.

As the first animal experiments with the Argentine wool fat suggested that this fat can assume the capacity of destroying the A-vitamin of other fatty substances, it was decided to heat the Icelandic wool fat and to feed it mixed with butter fat rich in A-vitamin. The method employed has been indicated by Fridericia (1923) in his experiments regarding the

capacity of some fatty substances to destroy A-vitamin. The fat is heated on flat trays under ample access of air to 102°C. for 42 hours. It thereby becomes a little harder, smells less of sheep's wool but becomes a little darker in color. With regard to the sulphuric acid reaction it behaves similar to the Argentine wool fat (see table 1), but there still remains a slight greenish tint, reminding one of the unheated fat. Iodine value 25.214, acidity value 6.90. The iodine value, thus, is unchanged. Later on another portion was heated in the same way for 42 hours, but to 110°C. This time it became considerably darker and smelled as if slightly burned. The sulphuric acid test now fails to give a green tint but only the brown precipitate. The fat thus treated was fed in continuation to the same animal which had received the fat heated to 102°C.; the outcome is discussed below.

Young pied rats previously fed on mixed food were used in the feeding experiments. The methods of Zilva and Miura (1921) for quantitative A-vitamin testing was followed. The vitamin-free food consisted of:

20 per cent purified caseinogen
5 per cent salt mixture
3 per cent purified agar
5 per cent dried top yeast (autolyzed)
5 per cent purified rice starch
15 per cent purified (oxidized) lard

The caseinogen is purified by letting it stand for one day in 96 per cent alcohol, then boiling it in the alcohol, then letting it stand for one day in ether, then again standing in 96 per cent alcohol for one day, and lastly drying it and heating it on flat trays for 24 hours to a temperature of 100°C. under abundant access of air.

The agar (pulverized) is purified by washing it with 96 per cent alcohol. The top yeast is autolysed for 24 hours at 37°C., then mixed with the same volume of purified rice starch and dried at room temperature.

The rice starch is purified with 96 per cent alcohol at room temperature for 24 hours, dried and ground. The pork fat is heated for 24 hours to 102°C. on flat trays under abundant access of air.

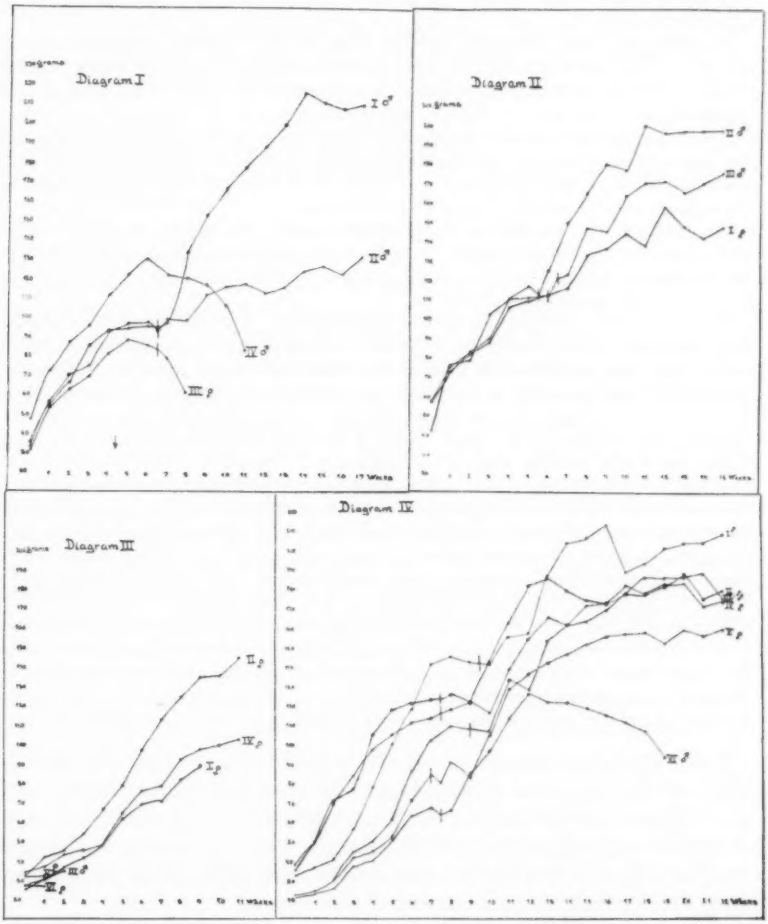
The salt mixture consisted of

10.38 grams sodium chloride
15.96 grams magnesium chloride
20.82 grams primary sodium phosphate
57.24 grams secondary potassium phosphate
30.40 grams primary calcium phosphate
78.00 grams calcium lactate
7.08 grams ferric citrate

The rats had free access to water. They were kept at a constant temperature of 20°C. in an almost dark room, completely shut off from daylight.

The above specified food is used in all A-vitamin experiments at this Institute and has proved to be quite free of A-vitamin.

Experiment I with Argentine wool fat (diagram I). Four young rats are used. They are first given A-vitamin free food. After 4 to 6 weeks they have ceased grow-



ing and in the seventh week they all begin to lose in weight. Then 1 rat receives 10 per cent butter fat (freed of casein, salts and water) mixed into its food *without* pork fat. The next rat receives 10 per cent mixed butter fat and wool fat to equal parts in the basal food and *without* pork fat. The next rat gets 10 per cent wool fat mixed into the basal food *without* pork fat. The fourth rat continues to get the basal food *with* 15 per cent heated pork fat. The first rat which receives the butter alone,

soon becomes better and grows in a normal manner. The second rat with mixed butter fat and wool fat grows slowly and by no means normally. The third which gets wool fat alone, dies of typical avitaminosis and the fourth dies likewise of typical avitaminosis. It thus appears that the wool fat does not contain A-vitamin and that it has partly been able to destroy the A-vitamin of the butter fat by being melted together with the latter or that it has, perhaps, in another way stunted the growth of the second rat.

Experiment II with Icelandic wool fat (*diagram II*). Three young rats are employed. After four weeks on A-vitamin free food, they have ceased to grow. After 6 weeks they all lose in weight and show eye symptoms and other signs of avitaminosis.

No. 1 then gets 10 per cent butter fat mixed in the basal food without pork fat. No. 2 gets 10 per cent wool fat mixed with butter fat to equal parts in the basal food without pork fat. No. 3 gets 10 per cent wool fat in the basal food without pork fat. All animals recover and grow in a normal manner.

According to this experiment it appears that *Icelandic wool fat contains considerable quantities of A-vitamin* and that it accordingly has not been able to destroy the A-vitamin of the butter fat, as the rat which receives both fats mixed together grows even better than the others.

Experiment III with Icelandic wool fat (*diagram III*). Six young rats are employed. With regard to these young rats it should be observed that their mother had for 6 weeks lived on completely A-vitamin-free food, before she littered. The young thrived well and appeared to be normal in every respect with the exception of one which had spastic paralysis in the hind limbs. After their birth the mother continued to get A-vitamin-free food. After 3 days she had eaten 4 of her ten young. Those remaining increase normally in weight and get nothing but the A-vitamin-free food and the mother's milk. After they have become 32 days old, they very suddenly all cease to grow and have all got incipient xerophthalmia. At this period they are taken from the mother and continue to get A-vitamin-free food. No. 1 (the one with the paralysis) gets 0.50 gram butter fat separately every day; it recovers at once, but not of the paralysis, and grows in a normal manner. No. 2 gets 0.25 gram butter fat separately every day, recovers, and grows in a normal manner. No. 3 gets daily 0.50 gram wool fat. For the first 2 weeks it increases in weight just as much as the others and the eyes get better, but it quickly develops enteritis (diarrhea) and dies. No. 4 gets daily 0.25 gram wool fat. It also develops enteritis but not so seriously as no. 3; it recovers and grows in a normal manner. Nos. 5 and 6 get no additional food and die after one week has passed with typical symptoms of avitaminosis.

From these experiments it may be concluded that *Icelandic wool fat contains such quantities of A-vitamin,* that even 0.25 gram daily is sufficient to cure a rat of 35 grams suffering from want of A-vitamin and sufficient for it subsequently to grow in a normal way. It appears furthermore that the digestive tract of the rats could not stand the wool fat and that they develop enteritis as a result.

Experiment IV with Icelandic wool fat (*diagram IV*). Six young rats are employed which have had A-vitamin-free food. After they have ceased to grow and have shown symptoms of avitaminosis, wool fat and butter fat are given mixed in the A-vitamin-free basal food *without* pork fat. No. 1 gets 10 per cent unheated wool fat; it recovers and grows in a normal manner. No. 2 and no. 3 get 5 per cent heated wool fat in the food without pork fat and are fed *separately* with 5 per cent butter fat; they

recover and grow in a normal manner. No. 4 and no. 5 get 5 per cent heated wool fat plus 5 per cent butter fat mixed into their food *without* pork fat; recover and grow likewise in a normal manner. No. 6 gets 10 per cent heated wool fat. It grows well at first but soon gets ill and loses much in weight, to eventually die of typical avitaminosis.

From these experiments it may be seen that *Icelandic sheep's wool fat contains considerable quantities of A-vitamin*, which can be destroyed by heating for 42 hours to a temperature of 110°C. under access of air; that the wool fat thus heated does not have a toxic effect on rats which receive at the same time butter fat, and that it has not destroyed the A-vitamin of the butter fat by being melted together with the latter, at all events not to a measurable extent.

The A-vitamin possibly originally present in the Argentine wool fat may have been destroyed in various ways. First, it is not known with certainty whether the wool had been subjected to cleansing processes or the like, but it is known for certain that it had been stored for a long time, for several years. The fat is distributed all over the wool and has an enormous surface. It is, therefore, to the highest degree exposed to the oxidizing action of the air. In this way the fat may have lost its A-vitamin and perhaps have acquired an A-vitamin-destroying capacity.

It seems, on the other hand, to be proved that the Icelandic sheep's wool fat, which was examined, contained A-vitamin in considerable quantities. No investigations exist concerning the vitamin content of Icelandic sheep's fat or mutton. But it is very probable that considerable quantities are to be found in the sheep in autumn. They have during the whole summer lived of green grass in sunny districts and have accumulated large quantities of fat reserves for the long winter. On the other hand several other factors could interfere with the A-vitamin contents of the wool fat. As pointed out above the fat is exposed to the oxidizing action of the air, but perhaps not so much so here in the cold climate as in the warm Argentine climate. What, however, would augment the relative amount of A-vitamin in the wool fat is the probability of a large part of the fat being saponified and washed away. The remaining wool fat then contains large quantities of non-saponifiable matter (in an experimental test one-third of the wool fat obtained was non-saponifiable) but as known the A-vitamin is contained in the non-saponifiable part. Furthermore the wool is exposed to the rays of the sun which, according to recent investigations, might augment its content of A-vitamin. Hess and Weinstock (1925) have shown that, by exposing cholesterol and phytosterol to ultra-violet rays, these substances can be changed in such a way that they acquired the antirachitic capacity. These authors also found that human skin and calf's skin could not protect rats against rachitis in amounts of 1.00 gram daily. But if the pieces of skin had previously been exposed to

ultra-violet rays for half an hour, the rats did not get rachitis; in the sheep's wool fat large quantities of cholesterine and isocholesterine are present as already shown by Schultze (1873) and later on by other investigators.

As it has been shown above by 3 corresponding series of experiments that Icelandic wool fat contains large quantities of A-vitamin the question arises: whence came this vitamin? Several possibilities exist. First, the A-vitamin may have had the same origin as the fat, i.e., from the fat glands in the skin. Secondly, the A-vitamin may have been formed under the influence of the rays of the sun on the otherwise vitamin-free wool fat. Thirdly, it might perhaps be thought that the A-vitamin came from the green grass in which the sheep lie when resting. In the present state of our knowledge of vitamin there is hardly any reason to imagine more possibilities. The third mentioned possibility seems to be very improbable. There was no sign of the wool being soiled by grass or the juices of the grass, which would have been apparent by a green color. The fat is exclusively found in the fine wool near the skin and would, thus, not come into direct contact with the grass when the sheep lie down. The second possibility seems to be admissible in the light of Hess and Weinstock's (1925) experiments which refer to an anti-rachitic effect, produced by light dissociated from actual A-vitamin production. The first possibility seems, however, most acceptable, i.e., that the vitamins have accompanied the fat itself through the fat glands in the skin. Little is known of the fate of the A-vitamin in the body. But Cooper (1923) has found A-vitamin in the urine of man and dogs receiving large quantities of A-vitamin in their food. The present experiments seem to show that *the A-vitamin is secreted through the skin.*

SUMMARY

1. Fat obtained by ether extraction of old "washed" Argentine wool yields no color reaction with sulphuric acid, but behaves like heated (oxidized) fats which, in other experiments, have been shown not to contain any A-vitamin, but on the contrary probably to possess an A-vitamin destroying capacity.

2. In animal experiments the Argentine wool fat was proved not to contain any A-vitamin but it was doubtful whether this fat was able to destroy the A-vitamin contained in butter fat.

3. Wool fat obtained from fresh Icelandic unwashed sheep's wool gave with sulphuric acid a green color reaction which disappeared when the wool fat was heated for 42 hours to a temperature of 110°C.

4. The Icelandic wool fat proved, in 3 corresponding series of animal experiments, to contain A-vitamin, in rather large quantities. The

A-vitamin was destroyed on heating, but it could not be proved that the fat thereby acquired the capacity to destroy A-vitamin in butter fat.

5. It is thought probable that A-vitamin is secreted through the skin.

To the Director of the Institute, Prof. Dr. med. L. S. Fridericia, I owe my most sincere gratitude, first for his having suggested the idea of my investigating this subject, and then for his expert directions during my work.

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THE INFLUENCE OF TESTES ON METABOLISM

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From the Research Laboratory, Parke, Davis and Company

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In the literature dealing with the glands of internal secretion there are many references to the relationship between the gonads and metabolism. As proof of this relationship is cited the well-known fact that animals are castrated to make them fat and better for food purposes. However, castration does not always cause an animal to become fat. Korenchevsky (1925) states that when the gonads are removed from animals about 60 per cent become fat castrates and about 40 per cent become thin castrates. He finds that fat castrates show a decreased rate of metabolism and thin castrates show a less pronounced decrease and in some cases an increase in the rate of metabolism. He offers as an explanation of the different ways in which castration affects the rate of metabolism in different animals the following hypothesis: "These results are due not only to the direct influence upon metabolism of the removal of the sexual glands but also to the secondary changes produced by castration in the thyroid, hypophysis, adrenals and pancreas, as well as to the varying degree of functional efficiency of these glands before castration."

Aub (1922) believes that the gonads play only a minor part in the control of the rate of metabolism.

Murlin and Bailey (1917) and Bailey (1921) review the important investigations which have been made with the object of determining how much castration effects the rate of metabolism. Loewy and Richter (1899), (1902) determined the metabolic rate in one male and one female dog before and after castration. The female dog showed a decrease of 15 per cent in the oxygen consumption in the period of $2\frac{1}{2}$ months to $3\frac{1}{2}$ months after castration. In the period from 4 to 6 months after castration the female showed a decrease in oxygen consumption of 20 per cent. The male dog showed a decrease in oxygen consumption following castration amounting to 14 per cent but it lost enough weight to offset this when computed in terms of cubic centimeters of oxygen per kilogram of body weight. Luethje (1902) studied four dogs from the same litter, 2 males and 2 females. He castrated one male and one female, leaving the other two normal as controls. He did not find any noteworthy difference between the metabolic rates of the castrated animals and those of the control animals, five months after operation.

Murlin and Bailey (1917) studied the effects of castration on two female dogs. One of these had been thyroidectomized a year previous to the experiment. In about five weeks' time following castration both dogs showed a decrease in metabolic rate of more than 14 per cent in terms of calories per hour per kilogram of body weight, the thyroidectomized dog decreasing 14.2 per cent and the other dog 17.5 per cent. This is an interesting result as it shows that in this one case at least the thyroid did not take on a compensatory increase of activity following the removal of the ovaries.

Aub (1922) cites Korenchevsky (1914) and Rovinski (1913) as authorities for the evidence that if the thyroid gland is removed castration is not followed by the same decrease of metabolism as when the thyroid is present, the theory advanced being that the removal of the gonads influences the thyroid in some manner so that its secretory activity is lowered thus lowering the rate of metabolism. In his more recent paper Korenchevsky (1925) states that he believes this is not a settled point.

EXPERIMENTAL PROCEDURE. In our study of the effects of castration on a male dog many hundred basal metabolic rate determinations have been made. A normal female dog has been used as a control and her basal metabolic rate has been determined each time after that of the male has been determined.

We have used a portable Benedict respiration apparatus with valves instead of the electric impeller, as modified by Roth (1922). This apparatus was arranged to record on smoked paper on a large kymograph. In this way greater accuracy and permanent records were obtained.

The dogs were connected to the respiration apparatus by means of a rubber sleeve. This is a modification of the rubber mask devised by Dr. Margaret M. Kunde (1923). Our rubber sleeve fits tightly on the muzzle of the dog making a joint which is practically air-tight. The muzzle is shaved every few days so that the rubber comes in contact with the skin, making a better joint than would be made if the hair were in the way.

Two healthy dogs about two years old and about the same size were selected. They were trained so that they would lie on the table and not move for an hour. This training required two months. At first several short periods of training were given daily and these periods were gradually lengthened. After two weeks very loose-fitting rubber sleeves were put over the muzzles of the dogs. These sleeves were gradually made tighter and tighter. Then the rubber sleeves were connected to the breathing tube of the respiration apparatus. In this way the dogs became accustomed to the apparatus. After it was seen that the dogs could be depended upon to remain quiet for a half hour, when attached to the apparatus, the real experiment was begun.

The dogs were kept in a well lighted and heated animal house. They

were fed just enough to maintain their weights as nearly constant as practicable. Feedings were given at about half past four and any food that was not eaten was removed half an hour later.

Usually the basal metabolic rate of each dog was determined twice a day. The rectal temperatures were taken so that only determinations should be accepted which were taken when the dogs were in a normal condition. The temperature of the laboratory where the determinations were made was maintained at 25 to 27°C. When the temperature was down to 25°C. the dogs were covered with a cotton blanket during the test period.

Previous to each test the dogs laid quietly for half an hour. The basal metabolism was determined from an 18 minute period following which the spirometer was weighted and the apparatus was observed for 6 minutes longer. If the rate of oxygen consumption for the 6-minute period was more than 10 per cent higher than the rate during the 18-minute period it indicated that there was a leak in the apparatus and that the test should be discredited.

The basal metabolic rate determinations were made twice daily on each dog for one month as a control period. Then the male dog was castrated. The wound healed quickly by first intention.

After a week the basal metabolic rate determinations were continued as before. They were made twice daily quite regularly for three months after castration. In the next month only a few tests were made because of urgency of other work. The following month, July, was so warm that tests could not be made because the dogs were panting. When the weather became cooler tests were made twice a day for several weeks at intervals until a whole year had elapsed since the castration. At that time the castrated male showed a decrease in basal metabolic rate of 38.2 per cent as compared with the rate before castration. At the same time the female control showed a decrease of 33.8 per cent. This slight difference between the two dogs was believed to be within the range of experimental error. Such being the case we had failed to demonstrate that castration of the male dog had reduced his basal metabolic rate. Some other factor must have caused the decrease. It could not be due to temperature changes for we had gone through a whole year and no seasonal variation appeared. We believed the decrease in basal metabolic rate was due to close confinement of the dogs so that they had not had any exercise. In order to determine that point we sent the two dogs out into the country where they were given the freedom of a large enclosure formerly used for a chicken yard. There the two dogs ran and played in the open air for 10 weeks. The female dog had become pregnant while in Detroit where several dogs were kept in the same house. She gave birth to six puppies 8 weeks after being sent to the country. The puppies were taken away so as not to interfere with the experiment. In 2 weeks she had apparently returned

to a normal condition so the dogs were brought back to the city. Basal metabolic rate determinations were made twice daily on each dog for four weeks. It was found that the basal metabolic rates of the two dogs had not materially changed during the 10 weeks' freedom in the country.

The data collected during the seventeen months of the experiment are shown graphically in figure 1.

The final results, 16 months after the castration of the male dog, show that the basal metabolic rate of the male dog has decreased 46.8 per cent when calculated in terms of calories per hour per kilogram of body weight. The female control shows a decrease of 38.8 per cent.

During the period the male dog has gained 15 per cent in weight and the female has gained 18 per cent in weight. It is more accurate to express

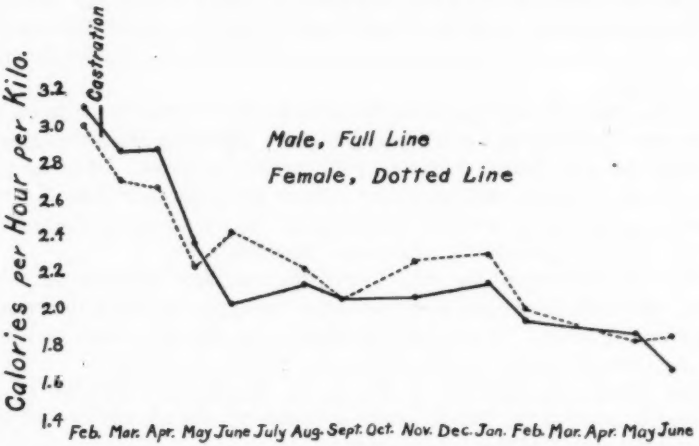

Fig. 1. Shows the variation in basal metabolic rates of the two dogs, the castrated male and the normal female.

the basal metabolic rate in terms of calories per hour per square meter of body surface. On that basis, the basal metabolic rate of the male dog has decreased from 59.2 calories per hour per square meter of body surface in February, 1924, to 33.2 calories in June, 1925, a decrease of 44 per cent. The female showed a decrease from 59.0 calories per hour per square meter of body surface to 38.1 calories, a decrease of 35.4 per cent.

Discussion. The castrated male dog shows only 8.6 per cent greater reduction in basal metabolic rate than does the normal female.

The effect of castration on the basal metabolic rate should have become demonstrable in sixteen months if such effect is of any considerable importance. Our experiment would seem to indicate that in this dog the testes did not have any very remarkable influence on the basal metabolic rate.

Korenchevsky (1925) points out that there may be considerable difference in regard to the influence of the gonads on metabolism in different individuals, for there are two types, fat castrates and thin castrates. In fat castrates the loss of the gonads is believed to be evidenced by a reduction in the catabolic phase of metabolism. For that reason the fat accumulates. In lean castrates on the other hand no such reduction in the catabolic phase of metabolism is believed to exist. In fact Korenchevsky (1925) states that metabolism in general may be even increased in such individuals.

Our castrated male dog should properly be classed as a "fat castrate" because he gained weight on a scanty diet. However, at the same time our female dog gained more weight than did the male. She was not a castrate as is shown by the fact that she gave birth to a litter of puppies.

It seems from this experiment that other factors play a much greater rôle than does castration in reducing the basal metabolic rate of animals under observation.

In the intervals between being brought to the laboratory for tests, our dogs remained quiet in a small animal house. They had almost nothing to interest or excite them. Also they got almost no exercise. It seems logical that under these conditions their psychic processes as well as all metabolic processes would be reduced in activity. In the same way the activity of the endocrine glands should likewise diminish.

We sent our dogs to the farm to exercise and play outdoors but when they came back their basal metabolic rates were approximately the same as before going away. It is likely, however, that changes which had been taking place for a year would not be overcome in ten weeks.

Our thanks are extended to Dr. E. M. Houghton, Director of the Research Laboratory, Parke, Davis & Company, for his advice and help during the course of this experiment.

SUMMARY

The basal metabolic rate was determined in a male dog before castration and then at frequent intervals until 16 months after castration. A female dog, not castrated, was used as a control.

The castrated male showed a reduction of 44 per cent in terms of calories per hour per square meter but the female in a similar way showed a reduction of 35.4 per cent.

We have failed to show that castration in itself reduces the basal metabolic rate.

We believe that under the experimental conditions other factors play a greater rôle in the reduction of metabolism than does castration. Factors to be considered are lack of psychic stimulation, lack of muscular exercise,

adaptation of the nervous system and endocrine gland system to a life calling for less metabolism than in an active normal life.

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THE CAPILLARY PRESSURE IN FROG MESENTERY AS DETERMINED BY MICRO-INJECTION METHODS

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For the purpose of studying some phases of capillary permeability the introduction of substances into single capillaries, using the micro-injection technique described by Chambers (1922), was attempted at the suggestion of Prof. M. H. Jacobs. It was first intended to study the rate of passage of certain substances through the capillary wall but the extreme variability of the results and the apparent importance of the condition of the circulation generally and locally has made it seem necessary to devise a method for determining the pressure in the particular capillary or capillary net during the period of injection.

The several indirect methods used to measure capillary pressure have produced a wide range of values, the determinations made for the capillaries of human skin alone varying from 75 cm. of water (v. Recklinghausen, 1906) to 10 cm. (Basler, 1914; Hill and McQueen, 1921b). Hooker and Danzer (1919), (1920) using a micro-tonometer found pressures between 18.0 and 26.5 mm. Hg.

These determinations have been frequently criticised since no correction is made for the pressure necessary to deform the superficial tissues, nor for the possible effect of total obstruction of flow. Liebesny (1923) believes blanching of the skin is due to the compression of the subpapillary venous plexuses, rather than to the emptying of capillaries. Apparently the values obtained by indirect methods are variable and difficult of interpretation as shown by the conflicting clinical observations on capillary pressure.

The first direct measurements of pressure in the microscopic vessels of the human skin were made by Carrier and Rehberg (1923), who introduced fine glass tubes, 10 to 20 micra in diameter at the tip, into superficial capillaries which had been made visible by an application of cedar oil, according to the technique of Lombard (1912). The pressure is determined by the level of the balancing water column at equilibrium. In the absence of any fixed mechanism to insert or hold the tubes, observations were limited to periods of a few seconds. Moreover, the size of

the tubes permitted measurements only in rather large dilated capillaries located in fairly resistant tissues, such as are found at the base of the finger-nail.

Apparently only indirect methods have so far been applied to the measurement of capillary pressure in the frog. Roy and Brown (1880) used a transparent, distensible membrane which was pushed against the tissue by a measured amount of pressure, while the vessels were observed under the microscope. This method has been criticised because flow was stopped and pressure thereby raised. L. Hill (1920) has used the same apparatus but regards the true value as that pressure which when suddenly applied will momentarily modify but not stop arteriolar or capillary flow. The pressures thus indirectly obtained were relatively low in the mesentery and web of the frog, 2 to 5 cm. of water, but somewhat higher in the kidney glomeruli and the tongue papillae, 5 to 10 mm. Hg.

The direct method here described has been used to measure the pressure in certain portions of the vessels in frog mesentery, in an effort to determine the average gradient of pressure fall through this particular portion of the peripheral circulation, and the possible relations of capillary pressure to certain factors such as flow, venous obstruction, and perhaps constriction. The pipettes used are 4 to 8 micra in diameter at the tip, and after being introduced into the capillary, can be held firmly in position over relatively long periods of time to measure the fluctuation of pressure in relation to other factors.

MATERIAL AND METHODS. The mesentery of the frog (Rana pipiens and small R. catesbiana) has been used throughout since it seems best adapted for micro-injection by the greater accessibility and visibility of the capillaries and larger vessels. A lateral incision is made through the skin with a cautery to avoid loss of blood, and the remainder of the abdominal wall cut by scissors. A loop of intestine is delivered through the incision and laid loosely over a transparent glass stage. A constant, slow drip of saline from a small reservoir on the frog board keeps the preparation moist and clean.

The microscopic implements consist of a blunt rod and the micro-pipette (fig. 2). The rod is made by fusing a small ball, 20 to 50 micra in diameter, on the end of a glass micro-needle. It is convenient to stop flow in a capillary temporarily or permanently by pressure, or to hold the mesentery firm during the introduction of the pipette into the more resistant larger vessels. The micro-pipettes are made of Pyrex capillary tubing with the small gas flame or micro-burner described by Chambers (1918). The tip is broken off against the edge of a glass slide until the diameter of the orifice is from one-sixth to one-third the length of a frog's red corpuscle, i.e., from 4 to 8 micra.

The movements of each implement are governed by a Chambers

micro-manipulator. It has been necessary to make some changes in the arrangement of the separate parts. The pipette and rod both come to the microscopic field from the right side of the stage, and the pipette is directed at a gentle slope downward instead of sharply upward as in a hanging drop preparation.

The other end of the micro-pipette is connected with a device for the application and measurement of pressure (fig. 1). This consists essentially of a reservoir, *R*, a syringe, *S*, for exerting large positive or negative pressures, and a column of water, *W*, by which measured amount of pressure up to 30 cm. of water may be applied to the contents of the

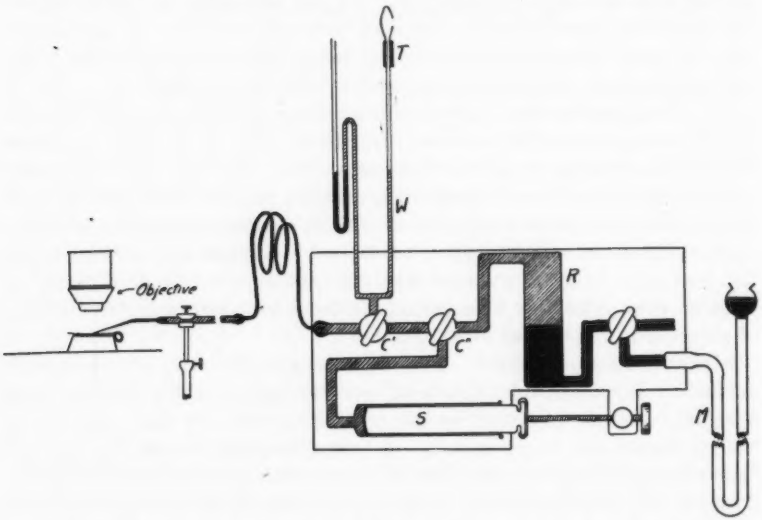

Fig. 1. Diagram of the apparatus used to measure pressure in single capillaries. For explanation see text.

micro-pipette. A dye solution may be drawn into the tip of the micro-pipette by a column of mercury, *M*, or by carefully withdrawing the plunger of the syringe when the two stopcocks, *C'*, *C''*, are properly arranged. For accuracy of control the plunger of the syringe is moved by a screw adjustment.

After the pipette has been introduced into the capillary the stopcocks are so turned that the pressure is applied to the dye in the pipette by the column of water, *W*, the height of which can be changed rapidly by the movement of the syringe plunger. To correct for capillarity in the upright tube the instrument has been calibrated by balancing the water column against known pressures.

For measuring the higher pressures found in the arteries a mercury column is more convenient. This is connected through a U-tube with the water manometer. If the opening at the tip of the tube, *W*, is open the whole system functions as a column of water, but if it is closed by a ringlet of rubber tubing, *T*, the pressure applied is measured by the mercury manometer providing correction be made for the column of water between the surface of the mercury and the tip of the pipette. This arrangement allows a more rapid succession of pressure determinations in arterioles and arteries than would otherwise be possible.

Photographs with a magnification of 80× have been made by a photomicrographic ocular permitting simultaneous observation and exposure.

Methods of measuring capillary pressure. Objections have been raised against the indirect values because of the blockage of flow, and apparently this same source of error must be guarded against even in single capillaries. If flow be stopped in a capillary at some point and the pipette is introduced on the arterial side of the obstruction the pressure there is some centimeters of water higher, depending upon the fall in pressure in in the region at the time of measurement, than on the venous side. Thus in figure 3 is shown a photomicrograph of such a capillary in which after occlusion the pressure on the arterial side was 15.0 cm. while on the venous side it was only 10.2 cm. of water. Hence in measuring pressure in any capillary, flow should not be stopped by the introduction of the pipette since the pressure then measured is not that of the capillary pierced but of the vessel with which it is connected. The error is apparently small if the vessel is a part of a network of short interlacing capillaries, but becomes greater in the long vessels connecting more directly arteriole and venule.

Two methods have been used in the determination of pressure in individual capillaries. The first is the more difficult but under proper conditions will measure both diastolic and systolic pressures in the vessel pierced. A very fine pipette, filled with a dye such as methylene blue, may be introduced into a dilated capillary or venous capillary and the flow not be hindered if the tip is brought as close as possible to the endothelial wall (fig. 4). When flow has continued for some minutes and is to all appearances normal the measurement may be made. As the pressure on the dye in the micro-pipette is gradually raised by increasing the height of the column of water in the upright tube, a point is reached where there is a barely visible spurt of dye during diastole. This would indicate the lowest pressure in the circulatory cycle,—diastolic pressure. As the height of the water column is further increased another point is reached at which the dye flows into the passing plasma continuously, though the amount is larger during diastole, and just perceptible during systole. This gives some indication of systolic pressure. The determination is

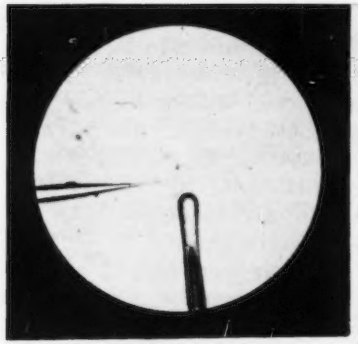

Fig. 2

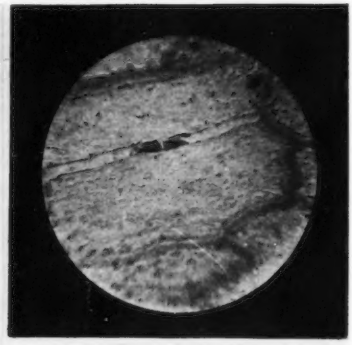

Fig. 3

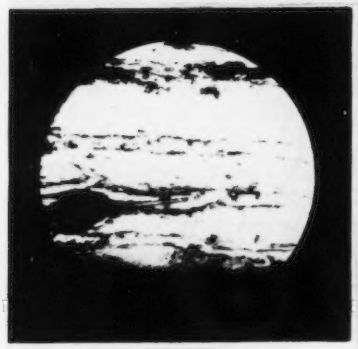

Fig. 4

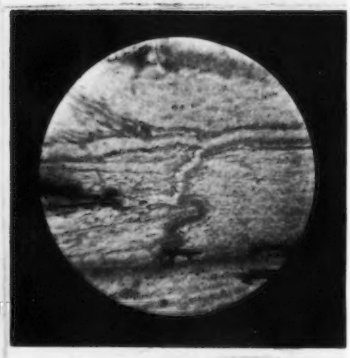

Fig. 5

Fig. 2. Photomicrograph (× 80) showing the micro-pipette used to pierce the capillaries and the glass rod for holding the mesentery or stopping flow.

Fig. 3. Photomicrograph (× 80) of capillary in which after stoppage of flow the pressure on the arteriolar side was 15.0 cm. of water, while on the venous side of the obstruction it was only 10.2 cm.

Fig. 4. Photomicrograph (× 80) showing the determination of systolic and diastolic pressures in a venous capillary by the introduction of a fine pipette close to the endothelial wall, thus avoiding any interference with flow. For the sake of contrast the corpuscles were washed out by a stream of saline from the pipette during the period of exposure.

Fig. 5. Photomicrograph (× 80) showing the method of determining pressure with uninterrupted flow by the use of a collateral capillary as a side tube for the insertion of the micro-pipette. Saline is flowing from the pipette and is passing into the vein. The pressure is being measured in one of the larger connections which resemble venous capillaries in size but pass directly from the arteriole to the small venule. The vessel into which the pipette was inserted had all the characteristics of a true capillary.

not always feasible and must be made with care since a too rapid flow of blood may dilute the dye and give falsely high results. The patency of the tip of the pipette must be frequently tested since a fold of endothelium, practically invisible under low power, may cover it and partially or completely obstruct flow.

The second method has been the more generally useful and is entirely accurate for measurement of mean pressure but permits only a rough estimation of pulse pressure. It requires the insertion of the pipette into some collateral of the capillary, the pressure changes of which are to be measured (fig. 5). The pressure in this occluded collateral will be equal to the lateral pressure in the straight vessel at the point opposite the opening. Flow proceeds uninterruptedly throughout the entire period in the capillary observed. After the pipette is introduced into the collateral a few corpuscles may be drawn out of the main stream back into the vessel functioning as a side tube. Intermittent movement toward or away from the pipette occurs synchronously with the heartbreat when the pressure of the water column is below or above mean pressure respectively. When the proper height is reached the dye-plasma junction, or the corpuscles, whichever is used to determine the point of equilibrium, will oscillate back and forth about a single point, thus giving the mean pressure in the capillary at the moment. With lowering of pressure, the corpuscles will move toward the pipette only in systole, remaining stationary in diastole. At a higher pressure the converse will be true; the movement is away from the pipette in diastole but ceases in systole. The difference between these two levels gives a general idea of the magnitude of pulse pressure, but in all probability not so accurately as the first method.

Although both a water column and a mercury manometer have been used all figures used in this paper have been changed to centimeters of water pressure.

OBSERVATIONS. *1. The capillaries of the exposed mesentery.* The term "capillary" has been here reserved for the vessels which are composed of a simple endothelial wall, and are in more or less direct relation with the finest arterioles. They have sometimes been referred to as arteriolar capillaries, in contrast to the venous capillaries. Frog mesentery contains in addition vessels whose diameter is little greater than that of the fully dilated capillary but whose walls are composed of endothelium enclosed by a thin coat of connective tissue. Frequently two or more true capillaries can be seen emptying into one of these vessels, which are apparently a part of the venous or collecting system, and for structural identification are referred to as venous capillaries, though physiologically their function and innervation are probably similar to those of the purely endothelial vessels, according to Krogh (1922) and Vimtrup (1922).

When the mesentery of a pithed frog is first exposed to the air usually all the vessels are constricted and flow is at a minimum. Within a few moments the arterioles begin to increase in size, and the capillaries, in the beginning invisible, appear at first to be constricted, but finally nearly all become more or less dilated.

If the frog has been anesthetized there is apparently a tendency for the capillaries to remain in the constricted state, either throughout their length or especially in the portions nearer the arterioles. In the pithed frog also after the preparation has been in use for some hours there is a tendency for constriction to recur. The reactions of inflammation develop very slowly indeed if the preparation is kept well moistened by a constant flow of saline, or Ringer's solution.

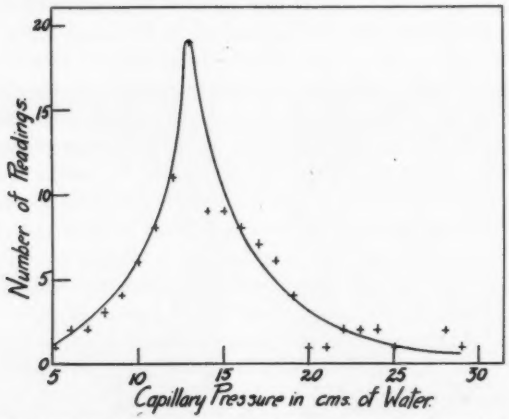

Fig. 6. Curve showing the distribution of 111 successive determinations of capillary pressure in the mesenteries of 21 frogs. Average pressure is 14.5 cm. of water.

The size of the capillaries in the exposed mesentery varies from 3 to 30 micra, with the majority about 20 to 25 micra in diameter when flow is active. The smaller ones by constriction may disappear entirely, the larger are merely reduced in diameter.

Most of the capillaries are undoubtedly relaxed, but the extent of dilatation found is usually not sufficient to be accompanied by that degree of permeability which allows the outflow of all the fluid portions of the blood, stated by Krogh and Harrop (1921) and Krogh (1922) to accompany maximum dilatation. The vessels are to all appearances functioning normally, since, though dilated, if properly moistened, they have been observed for hours without showing any tendency to stasis.

2. Range of pressure in the mesenteric capillaries. In a series of several hundred determinations of capillary pressures made in the mesenteries

of pithed frogs the values have ranged under different conditions from 5.0 to 29.0 cm. of water. Of these, 111 consecutive readings in the mesenteries of 21 pithed frogs have been charted in a frequency curve (fig. 6) in which over half lie between 10 and 15 cm. with 13 cm. as the most frequent.

The wide variation is probably due in part to the manner in which the measurements were made, purposely including all conditions of flow, from its complete absence, with or without plasma-skimming, to exceedingly rapid rates in some instances, in the hope of obtaining a fair average figure. Constricted and dilated capillaries alike have been tested and the values included.

In general the lower pressures, up to 10 cm., have accompanied very slow, or totally absent capillary flow, either with marked arteriolar constriction, or in constricted capillaries, irrespective of the condition of the arterioles. The very high values, from 22 to 29 cm., occur apparently with stoppage of venous outflow from any cause and if the condition is long continued it is accompanied by much reduced rate of flow, and a visible concentration of the blood ultimately resulting in stasis. In capillaries with the more average rates of flow, pressure lies between 10 and 22 cm. of water, the rapidity of flow increasing as the pressure rises, with a relation which seems quite close within certain limits, in dilated capillaries at least.

3. The fall of pressure in the mesenteric vessels. The gradient of pressure fall from artery to vein through the capillary bed has been of certain general interest. Since the work of Dale and Richards (1919) the intrinsic tone of the capillary wall has been regarded as playing a part in the consideration of peripheral resistance. Dale and Richards state (loc. cit., p. 63), "We find no evidence, however, to warrant the assumption that the steep fall of pressure in the arterioles suffers an abrupt flattening, becomes in fact horizontal, at a sharp line of demarcation between the smallest arterioles and the first capillaries. We find nothing to exclude the view that a general tone of the capillaries, if it exists, will play an important part in determining the peripheral resistance,. . . ."

To determine the average fall of pressure 189 determinations were made at various points in the peripheral vessels, 22 in the larger arteries near the attachment of the mesentery, 5 in the first bifurcations of these arteries near the intestine, 11 in the small arterioles, 111 in the capillaries, 17 in the venous capillaries, and 23 in venules and veins. Table 1 presents the averages of these figures along with the highest and lowest values observed in each group. Systolic and diastolic pressures, in centimeters of water, are given where pulse pressure was sufficiently definite to measure, otherwise only mean pressures are recorded.

These figures are all inclusive; no limitation was made except that the

mesentery be in normal condition at the time the pressures were taken and remain so during the entire period of measurement. Arterial pressures were determined by introducing a pipette with an orifice sufficiently large (30 to 40 micra in diameter) to allow red corpuscles to enter freely. Systolic pressure was measured with the mercury manometer at such a level that dye flowed out of the tube except at the height of systole, when two or three corpuscles would be pushed into the tip of the pipette. Then the pressure was lowered until an almost steady flow of corpuscles into the pipette was observed with a periodic stoppage or marked slowing of flow, thus giving a measure of diastolic pressure. Mean pressure, at which point there was not flow, but merely a fluctuation of the corpuscles back and forth through a single zone, was usually found to be about half way between the other two values, or slightly lower.

TABLE 1

Average pressures in the peripheral vessels of frog mesentery in centimeters of water

	ARTERY	ARTERY—FIRST BIFURCATION	ARTERIOLE	CAPILLARY	VENOUS CAPILLARY	VENULE AND VEIN
Diameter.....	0.25–0.30 mm.	0.12–0.15 mm.	40–50μ	10–30μ	30–45μ	0.25–0.35 mm.
Number of observations.	22	5	11	111	17	23
Highest value:						
Systolic.....	50	49	29	24	18.5	12.5
Diastolic....	24	25	17	20	17.5	
Lowest value:						
Systolic.....	24	35	6.5	5.0	6.7	4.5
Diastolic....	3	22				
Average:						
Systolic.....	39	38	22	14.4	10.1	7.5
Diastolic....	23	24	16			

Pipettes were also inserted at the first bifurcation of the artery near the intestinal wall and pressures compared with those of the artery at the proximal part of the mesentery. In every case there was practically no fall in pressure in that region of the peripheral circulation, though the entire breadth of the mesentery had been traversed, indicating the flatness of the pressure curve in the unbranching portion of the artery. Beyond this point the vessels are too close to the intestine to allow further measurement. The large venules and veins were pierced with the larger pipettes and their pressure measured by the same method used for arteries, except that the much reduced pulse pressure, amounting to only a few millimeters of water, made mean pressure the only significant figure.

It has not been so far possible to take these pressures simultaneously but only as rapidly as possible one after the other while flow and vessel

diameter were observed to remain practically the same. A protocol is given to show the times of the measurements and the variations in the readings for short periods.

PROTOCOL I

Young bull frog, of medium size pithed at 7:30 p.m. Abdomen opened without blood loss. Mercury manometer used throughout for all readings which have, however, been changed to centimeters of water.

7:47 Capillary of 20 micra diameter pierced and pressure determined
7:49 16.8 cm.—flow too fast to measure by eye since individual corpuscles were not visible
14.8 cm.—flow slower, about average speed
7:53 Terminal arteriole of 30 micra diameter pierced. It appeared to be of the same size as a dilated capillary but with thicker walls. It split into two capillaries of almost the same diameter within the microscopic field
7:54 Systolic pressure 26.0 cm. water
7:56 Mean pressure 21.0
7:57 Mean pressure 21.0
21.0
7:58 Larger arteriole, diameter of 45 micra entered
7:59 Systolic pressure 29.0 cm. water
30.0
Diastolic pressure 17.0
Systolic pressure 31.5
31.5
8:02 Systolic pressure 33.0
8:04 Pipette introduced into venous capillary of 35 micra
8:05 Mean pressure 12.0 cm.
12.5
8:06 Mean pressure 10.4
Pulse pressure was too slight to measure with a column of mercury

The fine tip of the pipette was now broken off till the orifice was about 35 micra in diameter.

8:16 Blunted pipette introduced into the vein which drained the region in which the previous pressures had been taken
8:17 6.5 cm. water
6.0 cm.
8:23 Entered large artery at the base of the mesentery
Systolic pressure 50.0 cm.
Diastolic pressure 25.0
8:24 Systolic pressure 45.5
Diastolic pressure 24.0
8:26 Systolic pressure 48.0
8:27 Mean pressure 36.0
8:29 Diastolic pressure 25.6
Systolic pressure 45.5
8:30 Diastolic pressure 25.6
8:32 Pipette introduced into the bifurcation of the same artery at a distance of 8 mm. from the first point

8:33 Systolic pressure 45.0 cm.
Diastolic pressure 24.5
8:34 Mean pressure 35.0

Frog still in good condition, flow is rapid, vessels well dilated, and there is no evidence of stasis.

The data obtained by experiments like the above, or others less complete, have been averaged (table 1) and plotted in the form of a curve in figure 7 in which the average length of each type of vessel forms the abscissa and the average pressure the ordinates. Below the curve are shown the diameters of the vessels plotted to scale. This represents therefore an average gradient of pressure fall in the peripheral circulation of the mesentery.

Virtually the fall of pressure presents wide variations under different conditions of arteriolar resistance, capillary pressure and flow. Five individual experiments have therefore been charted to the same scale as the average curve in figure 8 to show the types of gradients which are included.

With very low capillary pressures, as shown in the lowest gradient of figure 8 there is frequently no pulse pressure in the capillary, and little or none in the arteriole, the major fall of pressure occurring proximal to these vessels. Under these conditions the larger arteries of the mesentery are frequently contracted to such an extent that only two or three corpuscles can pass through a lumen that before was 0.15 mm. in diameter. The resistance has apparently shifted centrally due to the constricted larger vessels and the smaller arterioles and the capillaries have less importance in the determination of peripheral resistance. This condition is seen after application of adrenalin to the mesentery when arteriolar pressure is very low, and approximately equal to that in the capillaries as shown by direct measurement and as indicated by plasma skimming. Both Krogh (1921) and Froelich and Lak (1924) state that adrenalin has little or no effect on capillaries but constricts the arteries or arterioles above 0.1 mm. diameter. Resistance is also shifted centrally in the same way after hemorrhage when the mesentery may be almost bloodless, though the capillaries are widely dilated.

When the capillaries and arterioles are dilated and present the more rapid rates of flow on the contrary the fall of pressure may in extreme cases occur to a very large part between the capillary and the vein. In such instances as shown in the two highest gradients of figure 8, capillary pressure is very high, from 17 to 22 cm. of water, pulse pressure is increased, and frequently venous pressure is raised with a more than usually definite, though small pulse pressure of barely measurable magnitude.

In the average curve (fig. 7) and in the more moderate gradients of figure 8 there is a rather sharp reduction of pulse pressure between the arterioles and capillaries,—the usual finding with capillary pressures of

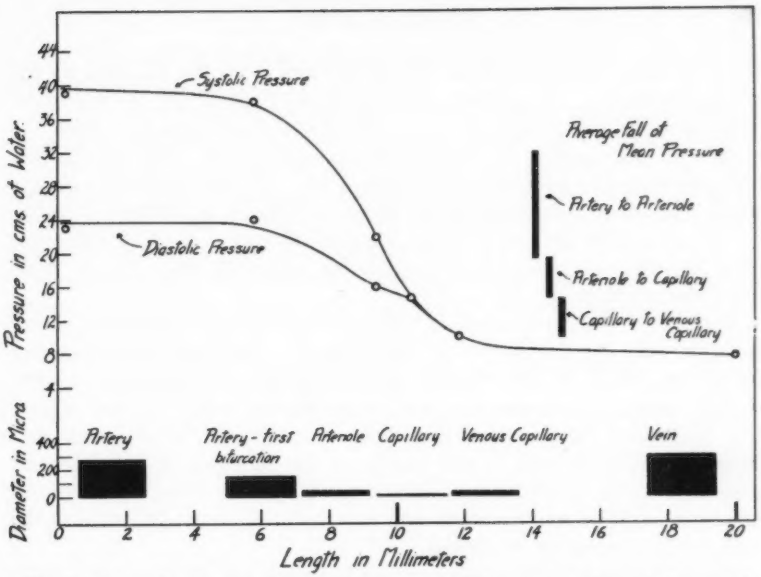

Fig. 7. Curve showing graphically the average fall in pressure through the vessels of the mesentery, using the data given in table 1. Pressure is charted in centimeters of water against the length of the respective vessels. Below the relative diameters of the vessels are shown in micra.

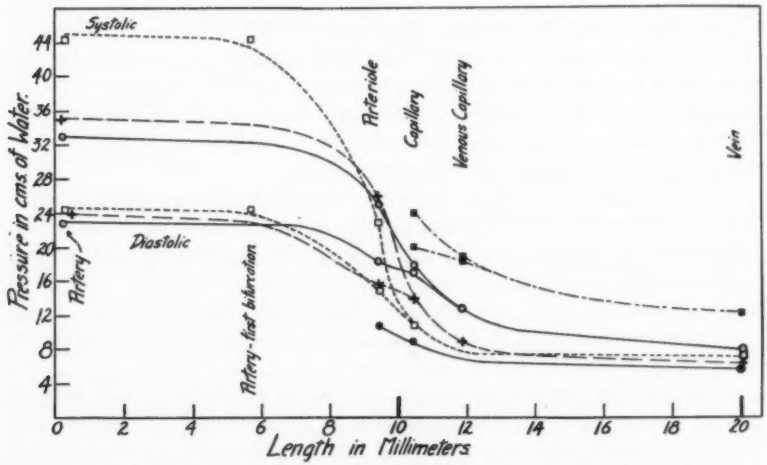

Fig. 8. Chart showing five pressure gradients determined in individual experiments in which pressures were determined one after the other as rapidly as possible, other things remaining constant. It shows the range of variation in the gradients forming the average curve in figure 7.

13 to 15 cm.,—and a moderate fall in pressure from the capillary to the venous capillary. If capillary pressure is less than 10 cm. a pulse pressure can only rarely be measured. At 15 cm. there is usually from 0.5 to 1.0 cm. difference in the systolic and diastolic levels, while at the very high pressures the difference may reach 4 cm. Except in the exceedingly rapid flow all over the mesentery there is little or no difference in the systolic and diastolic levels in the venule and vein. A slight difference may be made out in the venous capillaries, especially when flow is rapid and pressure high (fig. 8).

The rather sharp reduction in pulse pressure at the arteriole-capillary junction may be due partly to the changed conditions accompanying branching, partly to the narrow, at times almost slit-like openings frequently seen in the mesentery at the points where capillaries spring from the arterioles. Such narrowed orifices are believed by Richards and Schmidt (1924) to exist in the kidney at the junction of arteriole and capillary. With rapid rates of flow in the mesentery these openings can be observed to be widely patent and it is under such conditions that the higher pulse pressures are found.

There is noted both in the average gradient and also in two of the gradients of figure 8 a peculiar flattening of diastolic pressure in the region between the arteriole and the capillary. This has been noted very frequently, and in some cases the diastolic line may become almost horizontal in this zone. No explanation is offered at the present time. Speculation might include the possible effect of a velocity factor, or of a changed systolic pressure due to wave reflection in that region, or possibly also the presence of a special type of resistance, such as a slit-like opening between the arteriole and its capillaries, which would become more effective during diastole, and less so in systole since the force would then be sufficient to neutralize it to a certain extent by stretching.

Venous capillaries have given quite variable results, possibly because they are frequently hard to define, since a vessel which is obviously an arteriole at one extremity may within the same microscopic field receive capillaries and to all appearance become a venous capillary or even a small venule, with rapid flow, and a correspondingly high pressure. These have been mentioned by Hill (1921a) as short cuts forming a safety factor when blood stagnates in the capillaries.

4. Relation of pressure to velocity of flow in capillaries. The rate of flow in a capillary is apparently related to the gradient of pressure fall from capillary to vein, and a series of such gradients have been arranged in order of rate of flow in table 2. With reduction of the pressure difference between capillary and vein there is a reduction of the velocity of flow. At the average rate, somewhere about 0.5 mm. per second, the pressure difference between capillary and vein is usually between 7 and

8 cm. The fall in pressure through the same region as given in the average curve is 6.9 cm.

Capillary flow has long been known to be variable in character, differing not only in capillaries which lie side by side but also in the same capillary at different moments. It has been inferred that capillary pressure likewise changes from moment to moment, and such variations in the same capillary have been indicated by the work of Roy and Brown (1880) using the indirect method of measurement.

Direct measurement in a single capillary shows that pressure as well as flow is constantly varying and that the two apparently change in the

TABLE 2

Relation of flow to the fall of pressure from capillary to vein

	ARTERY	ARTERIOLE	CAPILLARY	VENOUS CAPILLARY	VENULE AND VEIN	PRESSURE FALL CAPILLARY TO VEIN
Rapid flow—moving so rapidly corpuscles cannot be seen individually			22.0	18.7	12.5	9.5
			22.0	18.5	12.0	10.0
	33/23	25/18	17.0	12.5	7.5	9.5
			18.0		7.5	10.5
			17.0		7.5	9.5
Moderate flow—about 0.5 mm. per second		27/17.5	14.0	7.0	6.0	8.0
	43/23		15.0		7.5	7.5
Slow flow—about 0.25 mm. per second		13.5	12.0		7.5	4.5
		18.0	15.0		12.5	2.5
		10.5	9.5		7.5	2.0
Very slow—barely perceptible with plasma skimming			8.0		7.5	0.5
No flow	25/20	6.5	6.5		6.0	0.5
		6.0	6.0		5.5	0.5

same direction providing the pressure in the vein remains approximately the same. Within the limits mentioned above high capillary pressures are nearly always found to occur in regions showing more rapid flow, and low pressures where flow is very slow or absent.

The relation between pressure and velocity may be measured by inserting the pipette into the side capillary, taking pressure as usual, then immediately afterward determining the time it requires for a corpuscle or group of corpuscles to pass from a certain point to the opening of the side capillary. It is possible to measure the pressure in the venule or vein only at the beginning and the end of the experiment, but this pres-

sure has been found to remain quite constant if capillary flow is increased over a small area only, as is usually the case.

Figure 9 shows the results of such an experiment plotted with velocity of flow and pressure against time. The chart shows the fairly close parallelism between pressure in the capillary and the flow in the same vessel. It indicates also the relatively large variations of pressure that may occur in a single capillary in successive moments. Again if 0.5 mm. per second be regarded as average velocity it required in this case a pressure difference of 6.5 to 7.5 cm. of water between the capillary at the point measured, near the arteriole, and the venule into which it

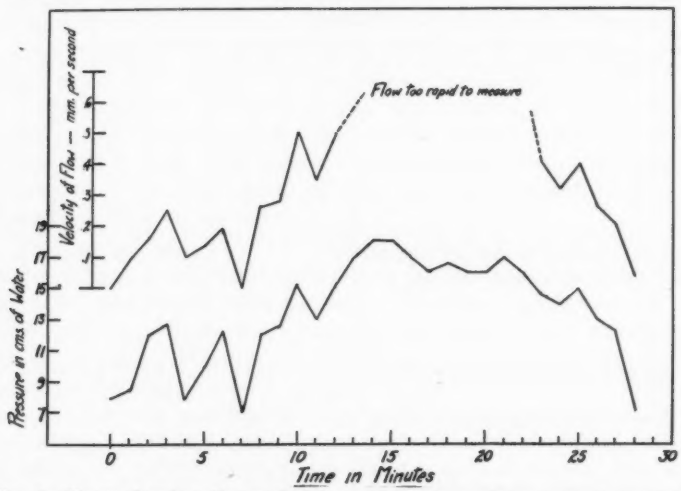

Fig. 9. Chart showing the variability of capillary pressure from moment to moment and the relation this bears to change of flow in the same capillary. Velocity was measured by determining the time required for a corpuscle to pass between two given points. Since estimations could not be made above 0.5 mm. per second with any fair degree of accuracy only the lower velocities are shown.

emptied, which had a pressure of 7.5 cm. This compares favorably with the average fall of 6.9 cm. obtained statistically.

Apparently average rate of flow usually accompanies a capillary pressure of 13 to 15 cm. when venous pressure is from 6 to 8 cm. As the capillary pressure decreases, venous pressure remaining constant, there is first noted a slower rate of flow, then in addition a reduction in the number of erythrocytes with increasing proportions of plasma, and finally plasma-skimming.

5. Plasma-skimming. Plasma skimming is most often seen in the presence of low capillary pressures but it seems to depend not so much

upon the level of pressure as upon the velocity of the stream of plasma passing from the arteriole to the capillary. If a widely dilated capillary be temporarily occluded and then gradually opened by slow elevation of the glass ball, plasma skimming occurs first, both leucocytes and erythrocytes passing by the capillary opening though a slow flow of fluid is passing through the partially occluded vessel as can be observed by following small fragments of cells which are nearly always present in the blood stream. As the obstruction is further lessened leucocytes can be skimmed off the axial stream and caused to flow through the partially occluded capillary. Finally, when entirely open, erythrocytes will be swept through as before the obstruction.

Frequently also when the heart rate is very slow a mass of corpuscles pass into a partially constricted capillary in systole, followed by a stream of plasma with relatively fewer corpuscles, or in extreme cases almost no cells, in diastole, apparently caused by the reduced stream when the arteriolar pressure falls during the latter period. Both of these are examples of plasma skimming through large orifices.

With normally high arteriolar pressure plasma skimming is also seen in constricted vessels, where frequently only a minute orifice at the junction of the capillary to the arteriole remains open. At intervals an erythrocyte can be seen to dart from the axial stream, plug the opening, and gradually squeeze through. On escaping this narrowed space it passes through the constricted vessel rapidly followed by plasma, until another erythrocyte is swept from the axial stream when again flow almost stops until the second cell has passed through the constriction. It is possible that some such occurrence may be the cause of the "stoszförmig" flow shown in the curves of Hürthle (1923), after the administration of adrenalin.

In the use of the collateral capillary as a side tube difficulty has frequently been experienced in causing corpuscles to leave the axial stream and enter the capillary, there to act as indicators of balance of pressure. The capillary in such a case is obstructed so that flow ceases, and pressure is equal to that of the arteriole. It apparently requires a lowering of the pressure in the micro-pipette of 1 to 2 cm. below that in the arteriole to cause corpuscles to enter the capillary. Such lowering of the water column causes plasma to flow into the pipette, and when this stream becomes sufficient a corpuscle or two will enter the capillary. Balance of pressure is then obtained by increasing the height of the column of water again until the corpuscle remains motionless. The more rapid the flow in the arteriole, and the smaller the entrance of the capillary, the greater the lowering of pressure must be.

Apparently plasma skimming though far more common with low pressures in the capillary or arteriole, may occur at any level, the sole require-

ment being a reduction in the stream of plasma passing into the entrance of the capillary. This may be accomplished by a small orifice which will reduce the stream of plasma though a fairly marked difference of pressure exists between the arteriole and its capillary. It may also occur with a large, dilated orifice if pressure is equalized by occluding flow in the capillary, as by the glass rod, or the micro-pipette which fills the lumen, in which case capillary pressure tends to approach the arteriolar level. It may also be observed with a large orifice after severe hemorrhage, or after the application of adrenalin to the mesentery, where constriction occurs in the larger arterioles, or the smaller arteries, above 0.1 mm. diameter as described by Krogh (1921) and others while the capillaries are dilated or unaffected, thus causing arteriolar pressure to approach capillary pressure, and reducing the flow of plasma and tending to emphasize the action of the larger, more direct channels leading from the arterial to the venous system.

6. Effect of venous occlusion on capillary pressure. The condition of the veins has been recognized as an important factor in determining the rate of peripheral flow and capillary pressure. Basch (1903) mentions resistance to outflow as one of the four factors determining capillary pressure. Hooker (1920), (1921) has emphasized the importance particularly of the small veins and Liebesny (1923) states that venous congestion constantly produces a marked rise of capillary pressure. According to Carrier and Rehberg (1923) capillary pressure follows venous pressure very closely with different levels of the hand.

If a single capillary is blocked by means of the microscopic rod so that its communications by collaterals are removed and it is connected only to its arteriole and venule, and flow is then stopped in the venous end, pressure rises rapidly to the arteriolar level, falling back to the lower value or in many cases slightly below, when the occlusion is removed.

Similar pressure changes, though smaller, are noted also with full collateral circulation. If flow in a capillary, venous capillary or even venule be shut off the pressure on the arterial side of the occlusion increases markedly for a few moments, then gradually lowers slightly to a level of 3 or more centimeters above the original. On removal of the obstruction it also falls below its first value, then climbs slowly back to the normal observed before circulation was stopped. An example is given under protocol II, in which a capillary was compressed 0.5 mm. from the arteriole.

PROTOCOL II

Pressure before occlusion	14.5 cm. water
Immediately after occlusion	20.0
After opening of adjacent capillary	17.0
Occlusion removed	13.5
Two minutes later	14.0

This may be repeated several times with similar results. The rise in pressure apparently depends on the rate of flow in the region, and the size and number of collaterals. The obstruction of a single fairly large venule results in a local rise of pressure of 3 to 4 cm., accompanied by a more rapid outflow of such a dye as methylene blue through the capillary wall, indicating an increased outflow of fluid from the vessel with the higher pressure. If pressure is above 20 cm. passage of fluid through the wall is rapid even in the venous capillary and a visible concentration of the corpuscles occurs. It appears from this and other experiments that fluid and certain dyes pass through walls of the venous capillary almost as easily as through simple endothelium. Krogh (1922) states that morphologically the venous capillary is very slightly different from the capillary itself and may well be a factor in the exchange of fluid.

7. *Effect of hemorrhage on capillary pressure.* After severe hemorrhage there is a marked vaso-constriction of the smaller and larger arteries in the mesentery. Capillary and arteriolar pressure is low, commonly 5.0 to 8.5 cm. and the number of corpuscles in the plasma of the capillary bed is much reduced, due to more or less marked plasma skimming. The activity of the channels which intervene between arteriole and venule is more evident, in spite of which venous pressure is usually lowered, the constriction more than compensating for this increased flow through the more direct communications.

8. *Pressure in capillaries of varying diameter.* Roy and Brown in their studies on pressure noted that an increase of external pressure caused a diminution of diameter of capillaries but never more than fifteen per cent, thus limiting the effect that internal pressure can have upon the size of the capillary lumen. Oinuma (1924) found that capillary size depends partly on blood pressure, partly on the condition of the capillaries themselves, but the relation of capillary dilatation to arteriolar dilatation while usually present was not constant.

Efforts have been made with this apparatus to measure the pressure in constricted capillaries in frog mesentery for comparison with those of dilated vessels. These have so far been consistently low, rarely above 10 cm. and never above 11, averaging for nine determinations 9.5 cm. The number obtained is small because of the difficulty of entering the vessels without clogging the very fine pointed pipette necessary. Certainly constricted vessels may have much lower pressures than dilated capillaries lying in the area adjacent and arising from the same arteriole. The proximal resistance in such a case could not determine the lowered pressure since the same arteriole supplies both nets of capillaries. If this is found to be consistently the case, that constricted capillaries have a lower pressure than dilated vessels, independent of arteriolar change it would seem to be an additional factor to be taken into consideration in

the explanation of the reduced rate of passage of substances through the walls of such vessels and possibly aid in explaining the reduced permeability of constricted capillaries as compared to dilated capillaries.

Spontaneous variations of pressure have been found to be accompanied by changes in diameter as in the following protocol:

PROTOCOL III

Pipette introduced into capillary, and pressure found to be

12.0 cm. water	
12.0	
12.0	Diameter 21 micra
12.5	

Gradually the flow increased in velocity till the corpuscles passed so rapidly that no individual cells could be seen. At this time pressure was 17.5 cm. Capillary diameter increased to 29 micra. The dilatation was obvious to the eye, even without measurement by the ocular micrometer. Coincidently pulse pressure was much increased over that at the lower level.

17.5 cm.	
17.4	
17.0	Diameter 27–28 micra
16.0	
15.0	
15.0	Diameter 24 micra
14.5	20–21
13.2	22
12.7	21
12.7	20
14.0	21
15.0	25–26
13.7	21
13.2	22
12.5	25

Finally stasis with a diameter once more of 29 micra.

Whether dilatation in such a case is the cause or effect of the change of pressure is entirely uncertain. Efforts have therefore been made to measure the pressure in a dilated capillary, to introduce from the pipette some substance, such as acid, or pituitrin, which should constrict the capillary while the arteriole remains the same, then to repeat the pressure determination. In some cases there is a definite fall of pressure coinciding with the constriction but the results are not sufficiently uniform to draw conclusions, though the tendency is definitely downward.

Measurements have also been made of pressure in capillaries under conditions producing increased flow and raised pressure coincidently determining changes in diameter of the vessels observed, to detect possible increase in size with rising pressure. The dilatation and increased rate of flow observed on the application of urethane to the mesentery is accompanied also by a slightly raised pressure as shown in figure 10. This

is independent of arteriolar change since urethane has no effect on any vessels but capillaries (Krogh, 1922). The dilatation may be measured and shows some correlation with the rise in pressure. The application of saline at a higher temperature to the mesentery results in a more rapid flow, dilatation of capillaries and higher pressure. Arteriolar changes may complicate this rise, however,

The evidence given is not definite enough to draw conclusions of any sort but apparently the tendency is toward a more rapid flow, higher pressure, and increased pulse pressure in more dilated vessels. This

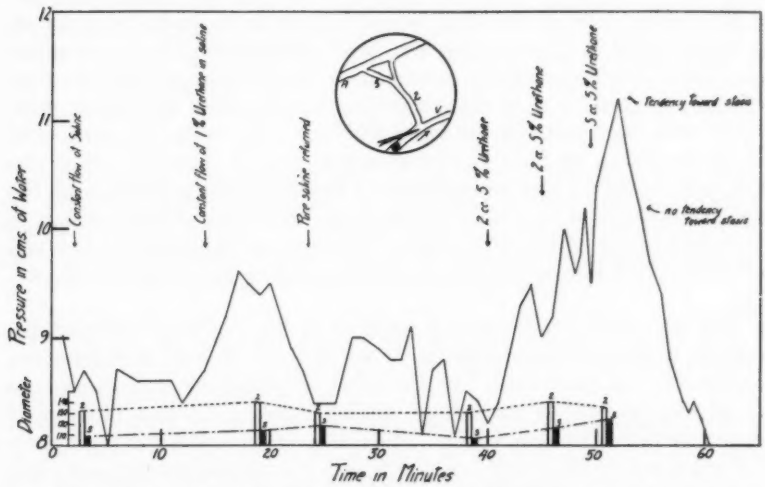

Fig. 10. Record of the changes in venous capillary pressure and capillary diameter after application of urethane to the mesentery. The inset shows the arrangement of the vessels. *A* was a small arteriole from which two capillaries rose to fuse into one, *2*, which emptied into the venous capillary, *V*, into which the pipette was introduced. The diameter of capillaries *2* and *3* were measured at intervals and the change in diameter is charted below in arbitrary units.

point will necessarily be considered in more detail in a later paper in relation to permeability, especially of constricted and dilated capillaries.

Discussion. The capillary pressures measured in the frog by Roy and Brown are generally conceded to be too high because of obstructed flow at the time of determination. A recent series of papers by Hill and McQueen (1921), (1922) detail the results obtained by the use of the same apparatus, by which pressure is applied over a large area of membrane, as the web or mesentery, or even a mass of tissue, as in the tongue or kidney. Pressure is applied to veins, venules, capillaries and arterioles alike. They record the pressure which, when momentarily applied to

all these vessels, is sufficient to modify but not to stop flow in the capillaries or arterioles. The pressures thus determined have been very low, 2 to 5 cm. of water in the mesentery and web, 5 to 10 mm. Hg in the tongue and kidney.

Venous pressure in the mesentery, according to the micro-injection method varies from 4.5 to 12.5 cm. of water with most values lying between 5 and 8 cm. A momentary stoppage or even reversal of flow in a few capillaries can be produced locally by but partially compressing one of the larger veins of the mesentery, with the glass ball. Since occlusion is only partial the pressure exerted is less than full venous pressure.

It would appear possible that momentary pressure producing a partial occlusion of all the veins in the area might slightly modify flow in a few capillaries without equalling even venous pressure, much less the true capillary pressure. It is also suggestive possibly that the lowest pressures have been found in the mesentery and web, both thin tissues in which the veins, by their greater diameter, project above the other vessels, and would be first compressed by the distended membrane of the apparatus, whereas in the masses of tissue, where less direct pressure is brought to bear on the veins, higher pressures are recorded,—5 to 6 cm. of water in the tongue papillae, and for the kidney glomeruli 5 to 10 mm. Hg.

The relationship between the pressure in the capillary field and the osmotic pressure of blood colloids for the frog is of interest with reference to the interchange of fluid. White (1924) has found the osmotic pressure of plasma proteins of three frogs ranging between 9.6 and 11.5 cm. of serum, or 10.0 to 11.9 cm. water. Thus the average pressure in the arteriolar end of the capillary is above, and that in the venous capillary is slightly below, the average osmotic pressure of the colloids as reported by White. Moreover capillary pressure changes continually, at some moments above, at others below, the reported colloid osmotic pressure, and flow is rapid or slow in the same relation. It is conceivable that in any capillary the flow of water through the wall might be outward at one moment, and inward at another, throughout the whole length of the capillary.

Apparently peripheral resistance, as suggested by Dale and Richards, is not limited to the arterioles alone. As the arterioles dilate the fall of pressure may be shifted more and more into the capillary area. On the other hand after hemorrhage, or after the application of adrenalin, the mesenteric circulation may be in large part cut out by the constriction of the larger arterioles and smaller arteries. Pressure is then at a low level in the capillary vessels irrespective of their condition, whether dilated or constricted, and the flow of blood is reduced, the mesentery becoming pale and bloodless. The dual resistance may have a local and general significance. The arteriolar resistance can apparently divert

much of the blood from the mesenteric circulation, thus increasing the blood volume passing to the remainder of the body, and diminishing the volume of the peripheral circulation. In the absence of such need for additional blood volume this resistance may be lowered, and capillary reactions would then be able to determine a local distribution to fulfill needs in the different portions of the mesentery or intestine. Such resistance would determine the high pressure and rapid flow in one capillary net, while an adjacent one arising from the same arteriole is composed of constricted vessels, with low pressure and reduced flow.

Further determinations of complete gradients under diverse conditions, local and general, will in all probability do much in clarifying the relationship between these two sets of resistance, the mere existence of which is here indicated.

SUMMARY

A method for the determination of pressure in individual capillaries by micro-injection is described. It permits the measurement of mean pressure, its fluctuations in successive periods, without disturbance of flow, and in certain cases it can be used to determine systolic and diastolic pressures in the capillary vessels of frog mesentery.

Capillary pressure in frog mesentery varies normally from 5.0 to 22.0 cm. of water, the large majority of determinations lying between 10 and 15 cm. Average pressure in the arteriolar capillary is apparently about 14.5 cm. and in the venous capillary 10.0 cm.

At the average rate of flow the fall of pressure in the peripheral vessels of the mesentery does not cease abruptly at the junction of the arterioles and capillaries, but continues to the venous capillary before flattening, though pulse pressure is sharply diminished at the junction of arteriole and capillary. The gradient varies markedly under different conditions of arteriolar resistance, capillary pressure and flow.

Pressure and velocity of flow in the capillary vary in the same direction and with rather close relationship. With an average flow of 0.5 mm. per second capillary pressure is usually about 13.0 to 15.0 cm. when venous pressure is 6.0 to 8.0 cm.,—a fall of 6 to 8 cm. from capillary to vein. The average fall through the same region determined statistically is 6.9 cm.

Pressure differs not only in different capillaries coming from the same arteriole, but also in the same capillary from moment to moment. Pressure may be above or below the reported osmotic pressure of plasma colloids throughout the whole length of any capillary in successive moments.

Occlusion of flow in a single capillary raises the pressure on the arterial side of the obstruction 3 or more centimeters even with fully open col-

laterals. Hemorrhage was found to reduce capillary pressure and increase plasma skimming. It is suggested that constricted capillaries may possibly have lower pressures than dilated vessels, independently of changes in the arteriole.

I wish to express my indebtedness and gratitude to Dr. M. H. Jacobs who suggested the application of the technique of micro-injection to the study of capillary permeability, and to Dr. R. Chambers for his kindness in demonstrating the use of his micro-injection apparatus and his method of making micro-pipettes.

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A STUDY OF BLOOD DIASTASES IN DEPANCREATIZED DOGS

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Many workers have concerned themselves with the function of blood diastases, with comparatively barren results. Carlson and Luckhardt (1909) believe that blood and lymph diastases are functionless, being by-products of organ metabolism which appear in the circulation on their way to destruction or elimination. Several conceptions militate against this view: In most animals the amount of diastase in the blood is greater than in an equal volume of blood-free liver, the pressure gradient of diastase concentration in the dog being from the pancreas to the blood, and from the blood to various organs (Macleod, 1913). Huber and Macleod (1917) confirmed the observations of Ishmura that a glycogen-like polysaccharide may exist in the liver sinusoids and sublobular veins of the rabbit. They found it present after puncture of the medulla. It is undoubtedly by means of blood diastase that this is transformed into sugar. This, theoretically at any rate, represents one function of blood diastase—to assist in the hydrolysis of glycogen in rapid glycogenolysis. It is important to note that such a function would not be detected by mere quantitative measurements of diastase in the blood. Similarly there is no change in the amount of glycogenase in the liver or blood in glycogenolysis consequent upon splanchnic stimulation, although it is practically certain that this enzyme is concerned in the transformation (Macleod, 1913). On the other hand, if blood diastase be important in the animal's economy, it is difficult to comprehend the utter disproportion that exists between the dietetic habits of the species, and the amount of this ferment in the blood. The diastatic power of the serum of the pigeon is very great, approaching that of parotid saliva in some of the herbivora, whereas goat serum has practically no diastatic power (Carlson and Luckhardt, 1909). There is no obvious difference in carbohydrate metabolism between these two animals. It is our belief that on the whole no one as yet has demonstrated that blood diastases possess any function, although because of the large quantities present in some animals, it is reasonable to suspect that one exists.

A fruitful method of investigating a problem in carbohydrate metabolism in the past, the employment of the diabetic organism is now doubly

valuable, in that by the use of insulin the normal state of the animal can be quickly, and if need be, permanently restored. It was hoped in the early part of this work that significant changes in concentration of blood diastase would occur following pancreatectomy, changes which the injection of insulin would consistently rectify. The problem assumed considerable importance in consequence of the communication of Myers and Killian (1917). These workers relegated to blood diastase the function of bringing about diabetic hyperglycemia. In blood diastase they found a mirror, as it were, of pancreatic efficiency. In clinical diabetes they reported an increased diastatic activity of the blood commensurate with the severity of the condition. Furthermore, as the patient improved under dietetic treatment, the diastatic power of the blood fell markedly. The hyperglycemia of nephritis they also found to be accompanied by a corresponding increase in the amount of diastase in the blood. As confirmation of their work, they reported that the diastatic power of the blood of *one* depancreatized dog was increased.

Using identical methods, Lewis and Mason (1920) completely failed to substantiate these clinical findings of Myers and Killian, and many workers deny that in depancreatized dogs there is any increase in blood-diastase.

From a careful review of the literature it has been impossible to come to any decision regarding the changes in blood diastase in depancreatized animals. Owing to the frequent use of depancreatized dogs in this laboratory, it has been comparatively easy for us to accumulate considerable data in this regard.

EXPERIMENTAL METHODS. It is an experimental fact demonstrated by many workers now that in the early stages of the hydrolysis of starch by diastase, the amount of sugar formed is not in accordance with the Schutz-Borrisov square-root law, but is a direct function of the enzyme concentration (Brown and Glendinning), (Chittenden and Smith, 1885). Thus C. Lovatt Evans (1912) in his study of the amyloclastic action of diastase, showed that when the ratio of substrate to enzyme is small, and the period of digestion so short that less than 30 per cent of starch is transformed, a linear relationship exists between the concentration of diastase and the extent of hydrolysis. With these principles in mind the following method was devised for estimating the diastase content of dog blood.

A 1 per cent solution of pure soluble starch is prepared containing 25 cc. 1 per cent NaCl and 25 cc. NaOH – KH_2PO_4 solution pH 6.7 (Clark and Lubbs) per 200 cc. Chloroform is added as a preservative, and the mixture kept on ice, to be discarded at the first sign of hysteresis. For a determination three tubes are placed in a wire basket. To each are added the following:

	(1)	(2)	(3)
Water, cc.	9.0	6.8	7.0
Plasma, cc.	—	0.2	1.0

The basket is placed in an ice bath for ten minutes. One cubic centimeter of starch solution is then added to tubes (1) and (2), which are then shaken briskly for a few seconds. The basket is then immersed in a water-bath kept at 37°C. for fifteen minutes, at the end of which time it is removed and placed in an ice-bath for three minutes. One cubic centimeter 10 per cent sodium tungstate followed by 1 cc. 0.66 N sulphuric acid are added to each tube. After mixing, the contents are filtered, the sugar in the filtrate being estimated by the Shaffer-Hartmann method. The amount of sugar present in 0.2 cc. plasma is deducted from tube (3). The amount of sugar (calculated as glucose) formed from 10 mgm. starch is large enough to be measured accurately, but small enough to be a direct function of the diastatic content of the blood. A blank analysis is always performed on the starch to rule out accidental salivary contamination.

Besides yielding figures whose fluctuations are directly proportional to corresponding variations in enzyme content, the method includes the precaution of keeping the reaction constant, which has not always been the case in the work of previous investigators.

In these experiments oxalated blood plasma was used. There is no objection to the use of whole blood providing the determination is made at once, and the incubation period is extended to thirty minutes, in order to yield enough reducing sugar for accurate measurement.

RESULTS. The diastatic power of a series of normal dogs was first investigated to see if this was at all constant, because it was considered that a constant concentration of diastase in the blood would indicate that the ferment fulfilled some specific function. The variations were found to be considerable, but the deviation from the average was usually small enough to be consistent with biological variation. The figures for all normal dogs investigated including those dogs that were subsequently depancreatized are collected in table 1. Some of the figures are presented again in subsequent tables to facilitate comparisons.

The effect of complete pancreatectomy was next studied in dog 1, a mongrel collie. The findings are synopsized in table 2.

Within 24 hours after pancreatectomy, the quantity of diastase in the dog's blood had diminished to half, and within five days it had dwindled to one-third.

Somewhat similar are the findings in dog A, a medium-sized fox-terrier.

On September 3, immediately before pancreatectomy, the diastatic power was 0.205 mgm. and the plasma sugar 0.086 per cent. On September 18, the diastatic power was 0.108 mgm., and the plasma sugar 0.694 per cent. On September 19, the diastatic power was 0.107 mgm., and the plasma sugar 0.670 per cent.

In consequence of statements appearing in the literature (Karsner, Koeckert and Wohl, 1921), (Myers and Killian, 1917), (Milne and Peters, 1912), that pancreatectomy in dogs leads to an increase in blood diastase, the next six dogs to be depancreatized in the laboratory were studied. The diastatic power just before pancreatectomy, and twenty hours after

pancreatectomy was observed. In each case there was a decided fall. The results are tabulated in table 3.

TABLE 1

Diastatic power of normal dog serum

NAME	SPECIES	DIASTATIC POWER*	PLASMA SUGAR	REMARKS
		mgm.	*per cent*	
Dog 1.....	Mongrel collie	0.149	0.153	Subsequently depancreatized
Dog A....	Fox-terrier	0.205	0.086	Subsequently depancreatized
Dog B....	Cocker-spaniel	0.196	0.089	Subsequently depancreatized
Dog Bu..	Cocker-spaniel	0.157	0.117	Subsequently depancreatized
Dog. Mr..	Fox-terrier	0.174	0.096	Subsequently depancreatized
Dog Ce...	Fox-terrier	0.133	0.100	Subsequently depancreatized
Dog F....	Fox-terrier	0.194	0.102	Subsequently depancreatized
Dog M...	Smooth-haired Irish terrier	0.255	0.112	Subsequently depancreatized
Dog P....	Collie pup	0.082	0.114	Subsequently depancreatized
	Husky	0.125	0.096	
	Irish terrier	0.139	0.101	
	Large fox-terrier	0.100	0.107	
	Black and tan	0.169	0.101	
	Fox-terrier	0.111	0.121	
	Collie pup (rachitic)	0.095	0.112	
	Fox-terrier	0.151	0.120	
Average.....		0.152		

* By this is meant the amount of reducing sugar formed on incubating 0.2 cc. plasma with 10 mgm. soluble starch as outlined above, deduction having been made for the sugar present in the plasma.

TABLE 2

Dog. 1. Effect of pancreatectomy

DATE	DIASTATIC POWER	PLASMA SUGAR
	mgm.	*per cent*
March 10.....	0.149	0.153
March 11.....	0.147	0.143
March 11.....	Animal depancreatized	
March 12.....	0.076	0.385
March 14.....	0.057	0.348
March 15.....	0.057	0.315
March 16.....	0.048	0.325
March 17.....	0.045	0.300

Inasmuch as the human diabetic in the vast majority of cases represents a partially depancreatized organism, two dogs were partially depancreatized in order to approximate the condition encountered clinically.

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ERRATUM

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Article by J. Markowitz and H. B. Hough, in table 3, page 575, dog P, the diastatic power 20 hours after pancreatectomy should read "0.052 mgm." instead of "0.152 mgm."

Dog B, a brown cocker-spaniel weighing 20 pounds was depancreatized on September 5. The whole pancreas excepting about 2 grams of the uncinate process, was removed. This latter with its vascular connections intact was brought out through an accessary incision in the abdominal wall to the right of the mid-line, and a little pouch was hollowed out for it in the subcutaneous tissues. The skin over it was incised to permit the escape of digestive juices.

On September 5, just before the operation, the diastatic power was 0.196 mgm., and the plasma sugar 0.089 per cent. On September 18 the diastatic power was 0.205 mgm., and the plasma sugar was 0.281 per cent. On October 1, the diastatic power was 0.139 mgm., and the blood sugar 0.460 per cent. On October 7, the subcutaneous remnant of pancreas weighing 1.5 grams was removed. On October 9, the diastatic power was 0.136 mgm., and the plasma sugar 0.690 per cent.

In this animal the blood diastase was at its pre-operative level for two weeks after the operation; eventually, however, it fell to the same level as in completely depancreatized dogs. This result is confirmed by the

TABLE 3

Effect of pancreatectomy

NAME	BREED	BEFORE PANCREATECTOMY		20 HOURS AFTER PANCREATECTOMY	
		Diastatic power	Plasma sugar	Diastatic power	Plasma sugar
		mgm.	*per cent*	*mgm.*	*per cent*
Bu.....	Cocker-spaniel	0.157	0.117	0.103	0.388
Ce.....	Fox-terrier	0.133	0.100	0.100	0.441
F.....	Fox-terrier	0.194	0.102	0.071	0.346
Mr.....	Fox-terrier	0.174	0.096	0.077	0.358
P.....	Collie pup	0.082	0.114	0.152	0.358
M.....	Smooth-haired Irish terrier	0.255	0.112	0.151	0.315

findings in dog G_4, a fox-terrier that was partially depancreatized on May 27. About one-eighth of the gland was left around the main duct. On a diet of 500 grams meat and 100 grams sucrose daily, pronounced glycosuria occurred. Three weeks after the operation the blood showed:

Diastatic power	Plasma sugar
mgm.	*per cent*
0.067	0.460

Even in the partially depancreatized organism there is, therefore, a decrease in the amount of diastase in the blood.

The attempt was now made to see if this enzyme ever came back to a normal level after pancreatectomy. It is possible by means of a suitable diet covered by insulin to restore all a depancreatized animal's functions to normal. If the animal be subjected to no strenuous experimental procedures even the reproductive functions may reappear—including oestrus

and pregnancy (Markowitz and Simpson, 1925). It is curious, therefore, to note that in animals kept alive and at ease for several months after pancreatectomy the concentration of blood diastase may attain a normal value. The results are presented in table 4.

The attempt was made to determine whether the administration of insulin to a depancreatized dog would have any immediate effect on the diastatic power of the blood. The results were variable. In a recently depancreatized dog the injection of insulin usually causes a slight rise, not, however, sufficiently great so that it approximates the pre-operative

TABLE 4

Blood diastase in animals kept alive with insulin. Blood removed 19 hours after last injection of insulin

NAME	DURATION OF PANCREATECTOMY	DIASTATIC POWER	PLASMA SUGAR
	months	*mgm.*	*per cent*
Dog 20	7	0.090	0.338
Dog 23	6	0.059	0.380
Dog 23	20	0.133	0.677
Dog Tr	6	0.189	0.613
Dog Lu	4	0.141	0.593

TABLE 5

Results in dogs kept alive and in good condition for several months after pancreatectomy (presented above in table 4)

DOG	BEFORE INSULIN		FOUR HOURS AFTER INSULIN	
	Diastatic power	Plasma sugar	Diastatic power	Plasma sugar
	mgm.	*per cent*	*mgm.*	*per cent*
Lu	0.160	0.593	0.141	0.028
23	0.140	0.677	0.133	0.189
Tr	0.194	0.613	0.189	0.040

value. In animals partially depancreatized, or depancreatized for several months, a slight fall in diastatic power usually results. At any rate, the findings are so inconstant as to make it exceedingly unlikely that the action of insulin is in any way fundamentally related to the presence of diastase in the blood. The following protocols, and table 5, show the experimental data in this regard:

Dog 1 (presented above under table 1), on March 17 showed a diastatic power of 0.045 mgm., and a plasma sugar of 0.300 per cent. Immediately after the blood was obtained, 10 units insulin were injected, followed in 3½ hours by an additional 5 units. Eight hours after the first injection of insulin, the diastatic power was 0.068 mgm. and the plasma sugar was 0.092 per cent.

Dog A (presented above in table 2), on September 19 showed a diastatic power 0.107 mgm. and a plasma sugar of 0.607 per cent. Three hours after the injection of 15 units insulin the diastatic power was 0.121 mgm. and the plasma sugar 0.092 per cent.

Dog B, partially depancreatized with subcutaneous graft as mentioned above, on September 18 showed a diastatic power of 0.205 mgm. and a plasma sugar of 0.281 per cent. Two hours after the injection of insulin, the diastatic power was 0.152 mgm. (duplicate analysis) and the plasma sugar 0.045 per cent. On October 1 the diastatic power was 0.139 mgm. and the plasma sugar 0.460 per cent. Three hours after the injection of 10 units insulin, the diastatic power was 0.133 mgm. and the plasma sugar 0.198 per cent. On October 9, two days after the removal of the subcutaneous pancreatic remnant, the diastatic power was 0.136 mgm. and the plasma sugar 0.690 per cent. Four hours after the administration of 10 units insulin the diastatic power was 0.098 mgm. and the plasma sugar 0.313 per cent.

. Dog G_4 was partially depancreatized as mentioned above. Three weeks after operation the diastatic power was 0.067 mgm. and the plasma sugar was 0.460 per cent. Three hours after the injection of 16 units insulin, the diastatic power was 0.058 mgm. and the plasma sugar 0.176 per cent.

Discussion. In these experiments which have been done on a sufficiently large number of animals to eliminate individual variations, it has been found that depancreatized dogs may show blood diastase values which, though much less than in the pre-operative condition, are nevertheless greater than those of other normal dogs. (Compare dog P, large fox-terrier and dog B in table 1, with dog B after pancreatectomy.) This observation, coupled with the one that the injection of insulin affects the blood diastases in an inconstant manner, render it exceedingly unlikely that blood diastases constitute an important mechanism in the production of diabetes or in the utilization of carbohydrate. Our experiments indeed would show that blood diastases have little if any function, were it not for the finding that after a primary fall in blood diastase in the depancreatized dog, there tends after some months to be a restoration to the pre-operative values. At any rate our work offers no experimental justification for the supposed significance of diastase estimations in the blood or urine in clinical diabetes.

CONCLUSIONS

1. In a series of sixteen normal dogs, the diastase content of the blood showed a maximum deviation from the average of 70 per cent.

2. Within 20 hours after complete pancreatectomy in dogs, there was invariably a great decrease in blood-diastase. In spite of this decrease the value in one instance was greater than in other normal dogs.

3. In one partially depancreatized dog the amount of diastase in the blood remained unchanged for two weeks after operation. Eventually the value fell as in the case of completely depancreatized animals. In another partially depancreatized dog the blood diastase was much diminished three weeks after operation.

4. Injection of insulin affected the blood diastases but slightly, and in a variable manner.

5. Three depancreatized dogs kept alive for many months by insulin showed blood diastase values which were practically normal.

6. No facts were elicited which would justify the employment of blood-diastase estimations in clinical diabetes.

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THE EFFECT OF THYROIDECTOMY ON SOME UNCONDITIONED RESPONSES OF THE SHEEP AND GOAT

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The characteristic somnolent attitude of the thyroidectomized sheep and goat suggests a profound alteration in the functioning of the nervous system, the nature of which has been the subject of an investigation in progress for some time. Our aim has been to study the establishment of temporary connections in the nervous system of the normal and thyroidectomized sheep and goat, a simple maze of three parallel alleys having been employed for the purpose (Liddell, 1923). The incentive to maze learning is twofold; the desire for food and the gregarious instinct. Having found its way through the labyrinth the animal is rewarded by being permitted, for a short time, to eat near a screen door through which it can see the flock in the barn.

Pavlov distinguishes two types of reflex, viz., conditioned and unconditioned. Unconditioned reflexes are inborn while conditioned reflexes are acquired. The seeking of the flock by a sheep isolated in the maze is an example of unconditioned response and the reaction to food is another. It is obvious that if data concerning the influence of thyroidectomy on habit formation are to be obtained one should first determine the effect of athyroidism on the incentives which are responsible for the formation of an habitual series of movements such as those performed by an animal in following the shortest path through the maze. Before communicating our data on habit formation it is, therefore, necessary to summarize observations which have been made from time to time concerning certain unconditioned responses of thyroidectomized sheep and goats since these activities are incentives to learning.

The reaction to food[2] has proved to be more inconstant in the cretins than

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[2] According to Pavlov's nomenclature the flow of saliva which results from the sight or smell of food is a natural conditioned reflex and appears early in development whereas the flow of saliva following the introduction of food into the mouth is an unconditioned response. The reactions to food here described refer to the animal's behavior in the feeding compartment where the grain in the food box is plainly visible. Such reactions should, perhaps, be designated natural conditioned reflexes rather than as unconditioned responses.

in normal animals. In the early stages of maze learning a normal animal frequently manifests great excitement and at such times refuses to eat. Later, it invariably feeds between trials. Cretin animals, however, continue inconstant in their response to the presence of food; sometimes eating greedily between trials, at other times nosing the food and at once

TABLE 1

Reaction to food of twin sheep, normal and thyroidectomized, after escaping from a short alternating maze

TRIAL	NORMAL SHEEP				CRETIN SHEEP			
	Choice	Time	Pauses in maze	Reaction to food	Choice	Time	Pauses in maze	Reaction to food
		seconds	*seconds*			*seconds*	*seconds*	
1 (r)	+	4	0	Ate at once	+	17	9	*
2 (l)	+	4	0	after every	−	25	12	*
3 (r)	−	12	2	trial	+	15	6	*
4 (l)	+	4	0		+	9	2	*
1 (r)	+	5	0		+	36	26	†
2 (l)	+	4	0		+	14	6	†
3 (r)	+	10	4		+	39	30	†
4 (l)	+	4	0		+	16	8	†
1 (r)	+	5	0		+	29	21	†
2 (l)	+	5	0		+	55	46	*
3 (r)	+	5	0		−	61	42	*
4 (l)	−	12	1		+	14	7	*
1 (r)	+	4	0		+	34	27	†
2 (l)	+	4	0		+	61	52	†
3 (r)	+	7	2		−	34	16	†
4 (l)	+	6	2		+	12	5	†
1 (r)	+	4	0		−	32	63	*
2 (l)	+	4	0		+	16	8	*
3 (r)	+	5	1		−	57	37	*
4 (l)	+	4	0		+	12	5	*

\+ Correct choice.
− Incorrect choice.
† Feeding.
* Not feeding.

turning away, or else ignoring the food box entirely. In some cases the cretin will stand before the food box for a number of seconds before beginning to eat. Occasionally, forcing the thyroidectomized animal's snout into the grain will cause it to eat when it has been standing over the food indifferent to its presence.

During one series of observations including 116 maze trials the cretin failed to eat in 27 per cent of the tests. In the maze employed in these experiments, shown in figure 3, the animal turns at once on emerging from the starting compartment and during the observations mentioned above the problem was that of simple alternation. Four successive trials per day were given and the correct turns for each day were right, left, right and left. The inconstancy of the cretin's behavior in the presence of food is well shown in tables 1 and 2 which include the complete data from the tests. In every case the normal sheep ate at once upon reaching the feeding compartment. In the first four trials of table 1 the cretin refused food after every test although its reaction in escaping from the maze was prompt and it turned correctly in three of the four trials. The next day it ate after each test. An inspection of the time values for these trials shows that the cretin's reaction to food is not to be correlated with its promptness in escaping from the maze, neither can the food reaction be related to right or wrong choices. It might be suspected, however, that the complicated nervous activity involved in choosing which way to turn is responsible for the cretin's capricious reaction to food. In table 2 data are presented from a series of trials in which choice is not involved. By means of a system of gates the animal on emerging from the starting compartment is compelled to make the correct turn which alternates from right to left as before. In this table the same irregularity in the feeding reaction is observed. The detailed notes of the cretin's behavior in the feeding compartment show that even when the animal ate between trials its behavior toward the food was inconstant.

The second incentive to maze learning, the gregarious instinct, is never demonstrably weakened following the extirpation of the thyroid glands. It has been repeatedly observed that cretinoid sheep and goats will attempt to follow the movements of the flock in the pasture. When the animals are in rapid movement it is usual to see the weakest of the cretins following in a group in the rear of the flock. Or, in some cases, two or three cretins, too weak apparently to keep up with the others, may be found grazing together far away from the rest. On one occasion a thyroidectomized sheep which could scarcely keep its feet was on its way in from the pasture many minutes after the flock had reached the pen. Another cretin sheep, also unable to keep up with the flock but far in advance of the other animal, waited a number of minutes for the weaker one so that both entered the pen together. While waiting it bleated repeatedly and the other replied.

It has often been observed that a cretin which exhibits extreme sluggishness in escaping from the maze, pausing repeatedly from one to two minutes, will at once approach the screen door and paw at it in the attempt to gain access to the flock and these attempts will increase in vigor if the flock begins to leave the barn. Even when the cretin remains in the start-

TABLE 2

Reaction to food of twin sheep, normal and thyroidectomized after escaping from a short alternating maze (forced turns)

NORMAL SHEEP				CRETIN SHEEP				
Trial	Time	Pauses in maze	Reaction to food	Trial	Time	Pauses in maze	Reaction to food	Behavior in feeding compartment
	seconds	*second*			*seconds*	*seconds*		
1 (r)	4	0	Ate at once after every trial	1 (r)	29	20	†	2 seconds pause at food box before eating
2 (l)	4	0		2 (l)	13	5	†	5 seconds pause at food box before eating
3 (r)	5	0		3 (r)	16	7	†	10 seconds pause at food box before eating; turned away from food once before eating
4 (l)	4	0						
				4 (l)	23	16	*	16 seconds pause at food box turning toward, then away from the food before going to the screen door between barn and maze
1 (r)	5	0						
2 (l)	4	0						
3 (r)	6	0		1 (r)	19	10	†	8 seconds pause. It nosed the food then turned away before eating
4 (l)	4	0						
				2 (l)	18	12	*	Walked directly to the screen door
1 (r)	5	0		3 (r)	19	10	*	Walked directly to the screen door
2 (l)	4	0		4 (l)	11	3	†	3 seconds pause before eating
3 (r)	6	0						
4 (l)	6	0		1 (r)	17	9	*	Walked directly to the screen door
				2 (l)	10	3	†	2 seconds pause, turning toward then away from the food before eating
1 (r)	4	0						
2 (l)	4	0		3 (r)	26	20	†	3 seconds pause before eating
3 (r)	5	0		4 (l)	13	6	†	Ate at once
4 (l)	5	0						
				1 (r)	22	12	*	4 seconds pause at food box, turning toward the food then rapidly away. Finally went to screen door
1 (r)	4	0						
2 (l)	5	0		2 (l)	13	4	†	2 seconds pause before eating. Soon went to screen door
3 (r)	4	0		3 (r)	13	5	†	5 seconds pause before eating, turning toward then away from the food twice
4 (l)	5	0						
				4 (l)	13	5	*	14 seconds pause near food box. Went to the screen door without having looked toward the food

				1 (r)	14	6	†	4 seconds pause before eating
				2 (l)	14	4	†	10 seconds pause before eating, turning toward then away from the food
				3 (r)	14	2	†	1 second's pause before eating
				4 (l)	14	7	†	5 seconds pause before eating, turning toward then away from the food twice

* Not feeding.
† Feeding.

ing compartment for as long as half an hour or has finally to be driven through the maze, when it reaches the feeding compartment and can see the flock it invariably goes to the screen door and makes persistent attempts to enter the barn. If the wooden storm door between the feeding compartment and the barn is closed, the animal, although its previous behavior has been lethargic, soon becomes excited and moves nervously about seeking escape from the feeding compartment. If the wooden door is then opened so that the flock is again visible the animal may once more begin eating.

An unconditioned response which has been utilized by Bekhterev and his numerous associates in the investigation of conditioned reflexes is the defensive movement which results from the stimulation of the skin of the foreleg with faradic current. It was deemed advisable to supplement the investigation of maze learning in thyroidectomized sheep and goats by the employment of the motor conditioned reflex method of Bekhterev in view of the following considerations. One of the earliest signs of experimental hypothyroidism is muscular weakness. Since locomotion is necessary in solving the maze problem it is possible that the differences in maze behavior between the normal and cretin animals may depend upon peripheral factors. For instance, the weakened muscles of the thyroidectomized animal may initiate defective proprioceptive impulses and in this manner modify behavior in escaping from the maze. Before one can be certain that the activities of the cerebral cortex in the sheep and goat are altered by the extirpation of the thyroid it is necessary to employ a method for the study of cerebral function which does not depend upon locomotion. The methods for the establishment of salivary and motor conditioned reflexes developed in the laboratories of Pavlov and Bekhterev satisfy this requirement. For our purpose the salivary reflex method seemed preferable because the effector of the reflex arc is glandular tissue and not muscle and, moreover, an accurate measure of reflex activity is obtained by counting the number of drops of saliva secreted. A parotid fistula was established in a cretin goat and the attempt was made to build a conditioned reflex to tactile stimulation. However, a more or less constant secretion of saliva accompanied by chewing the cud rendered this method impracticable. For example, during the interval between applications of the combined conditioned and unconditioned stimuli saliva was secreted from the left parotid duct at the following rates in successive minutes: 13, 13, 14, 13 and 15 drops. During the next rest interval the secretion rate in drops per minute was 16, 18, 14, 13, 16 and 10 drops in successive minutes.

Following the procedure outlined by Beritoff (2) we found that motor conditioned reflexes both to auditory and tactile stimuli could readily be established in the sheep and the goat. Since with this method the formation of temporary nervous connections depends upon the unconditioned

defense reaction of the leg to noxious stimulation, the effect of thyroidectomy upon this unconditioned response demands investigation. It was discovered that a defensive movement of the forelimb could invariably be evoked in the cretin sheep and goat provided faradic current of sufficient intensity was applied to the shaved skin of the limb. The threshold intensity, i.e., the strength of current just sufficient to initiate the unconditioned response was determined for a number of cretins and their controls. These data will be presented later. In the present communication we are concerned with the relation between the conditioned and unconditioned motor responses of the thyroidectomized animal and this relation was investigated in the following manner.

A cretin sheep and its twin control were selected for the preliminary

Figure 1

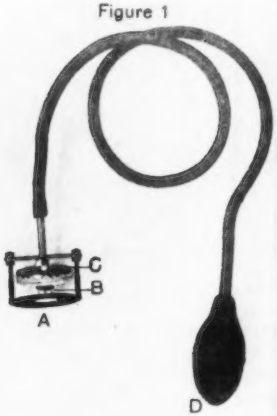
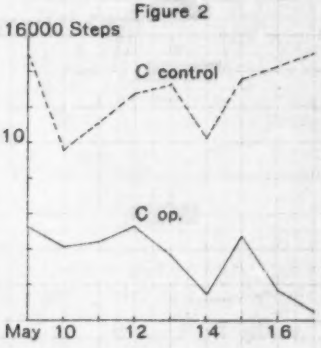


Figure 2



experiments. The wool was shaved from the rump and a spot on the skin tatooed. A tactile stimulus was applied to this spot by means of a device similar to that used in Pavlov's laboratory (see fig. 1). It consists of a tambour, *C*, to the rubber membrane of which is cemented a wooden block bearing five pins, *B*. The tambour is mounted on a frame attached to a perforated brass disc, *A*. This disc is fastened to the skin with a cement composed of resin, beeswax and ferric oxide, and the pins just above the perforation in the brass disc are caused to touch the tatooed spot on the skin when a bulb, *D*, connected with the tambour by a rubber tube is pressed by the observer. The unconditioned stimulus, a tetanizing faradic current, was applied to the skin of the forelimb through a leather bracelet wrapped with brass wire. The conditioned tactile stimuli were delivered at the rate of thirty per minute and preceded the electric shock

by an interval varying from two to ten seconds. The tactile stimulus was applied at the moment of the shock as well. To avoid the development of a time reflex the rest intervals between the combinations of conditioned and uncontitioned stimuli varied from thirty seconds to several minutes. During the experiments the observer was not visible to the animal. The first definite leg movement occurred in response to the tactile stimulus applied to the rump at the tenth combination of conditioned and unconditioned stimuli in the case of both the cretin and its control.

Motor conditioned reflex experiments were begun on March 26, 1925. On May 8 the cretin showed definite signs of muscular weakness and lethargy and the next day the subcutaneous injection of thyroxin was begun but without benefit. The animal became progressively weaker and more

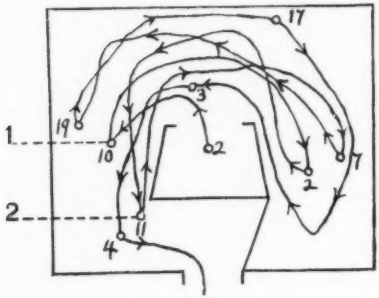


Fig. 3

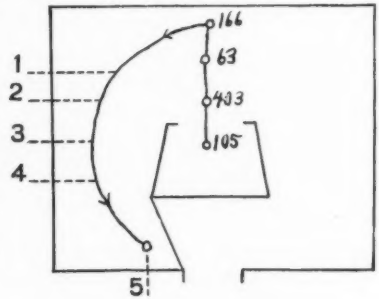


Fig. 4

Fig. 3. The effect of firing a revolver on the behavior of a normal sheep in the maze. Circles indicate pauses measured in seconds. *1*, Clicking empty revolver. Animal startled. *2*, Shot fired. Animal jumped.

Fig. 4. The effect of firing a revolver on the behavior of a thyroidectomized sheep in the maze. Circles indicate pauses measured in seconds. Shots fired at positions indicated by *1*, *2*, *3* and *4*. No effect on the animal's behavior. *5*, Animal driven out of the maze after 15 minutes.

somnolent and a pronounced decrease in spontaneous activity was observed (see fig. 2). Finally by May 14 it had difficulty in standing and would remain for half an hour in the maze without movement. The effects of various auditory stimuli were tried when the animal did not leave the starting compartment of the maze. For example, coughing and whistling were responded to by ear movements but locomotion was not induced. Repeated firing of a 32 calibre revolver directly back of the animal produced either no reaction or only the raising of the head and ear movements.

The effect of firing the revolver on the animal's locomotion in the maze was tried. The gun was fired from the observer's window in the second story of the barn. The result of this experiment is shown in figures 3 and 4. In figure 3 the clicking of the empty revolver startled the normal sheep

in the maze while the firing of a blank cartridge caused a sudden start followed by rapid movements about the maze and great timidity in entering the feeding compartment. As can be seen in figure 4, the firing of

TABLE 3

The effect of thyroidectomy on tactile conditioned reflexes in the sheep

\+ Motor response c.s. conditioned stimulus (tactile)
− no motor response u.s. unconditioned stimulus (faradic current)

DATE	NORMAL TWIN WETHER		THYROIDECTOMIZED TWIN WETHER	
	Response to conditioned stimulus	Response to unconditioned stimulus	Response to conditioned stimulus	Response to unconditioned stimulus
May 15, 1925.	+	+	+	+
	+	+	+	+
	*	+	*	+
	+	+	−	+
	+	+	−	+
	+	+	−	+
	+	+	−	+
	*	+	*	+
	+	+	+	+
	+	+	−	+
	+	+	−	+
	+	+	−	+
	*	+	*	+
	+	+	−	+
	+	+	+	+
	+	+	−	+
	+	+	−	+
	+	+	+	+
	+	+	−	+
	+	+	+	+
May 16.....	+	+	−	+
	+	+	−	+
	+	+	−	+
	+	+	+	+
	+	+	−	+
	+	+	−	+
	+	+	−	+
	+	+	−	+
	+	+	+	+
	+	+	−	+

* c.s. just preceding u.s.

four successive shots did not affect the cretin which continued its lethargic progress in the maze until it reached the closed gate of the cul de sac where it remained until driven out of the labyrinth.

On May 15 the cretin was too feeble to walk and was carried to the experimental room and hoisted into the harness. In the upright position it did not hang in the straps but supported its weight with head held up and eyes open. In seven trials on the preceding day the conditioned motor response to the tactile stimulus was obtained in every case while on this day the conditioned reflex had almost disappeared, as can be seen in table 3. In every case the application of faradic current of the strength which had been employed in forming the reflex, viz., the current from a Harvard inductorium with the secondary coil 4 cm. from the primary and with one dry cell in circuit, elicited a prompt leg movement. (The threshold current intensity at the beginning of the experiments was obtained with the secondary 6.7 $\pm$ 0.4 cm. from the primary.) The next day the experiment was repeated, the animal being still weaker. The results are also presented in table 3. Once again, the unconditioned response was promptly obtained at every trial but the conditioned reflex appeared only twice in ten trials. At the end of the experiment the animal was helped from the table and went to the corner of the room where it usually fed from a pail of oats. On this day it stood with head lowered and snout almost in contact with the grain but it did not eat even when a handful of oats was pressed against its mouth. It soon lay down and had to be carried from the room where it remained by the door from noon until 6 p.m. At this time it was found lying in the same position with head erect and eyes open. It was carried to the barn and was found dead the next day.

Discussion. The thyroid hormone may have a specific action on the nervous system or it may influence nervous function only through its effect on metabolism. A number of considerations seem to favor the second notion. In the absence of the thyroid a lower metabolic rate obtains. It has been mentioned elsewhere (Liddell and Simpson, 1925) that in thyroidectomized sheep and goats recurrent periods of muscular prostration and lethargy are observed. This would suggest an accumulation of products of faulty metabolism leading to depression, followed by their elimination leading to recovery. Recovery also occurs with striking suddenness when thyroxin is administered and this substance is known to increase the metabolic rate in hypothyroidism.

Lowered metabolism due to other causes may affect the higher functions of the nervous system. The nervous effects of long-continued undernutrition are in many ways similar to those observed in our thyroidectomized animals. Froloff (1925) has shown that in the slowly starving dog the activities of the cerebral cortex are profoundly affected. Well-established salivary conditioned reflexes are abolished and new ones cannot be formed. Somnolence supervenes and cannot be dispelled by the means which usually prove effective in combating this condition. However, the uncon-

ditioned salivary reflex persists until death just as did the unconditioned motor response in the moribund cretin sheep.

Whether the lethargic behavior of the cretin is due to somnolence or not is a matter difficult to decide. "Inhibition" says Pavlov (1923), "is partial sleep, or sleep distributed in localized parts, forced into narrow limits; true sleep is a diffused and continuous inhibition of the whole of the hemispheres." Perhaps diffuse inhibition induced by the faulty metabolism of the athyroid condition of the cretin sheep and goat but less widespread than in the case of sleep may account for the lethargic and unstable reactions (Liddell, 1925) observed in these animals. Further motor conditioned reflex experiments now in progress may throw more light on this problem.

SUMMARY

1. Since the formation of temporary nervous connections depends upon certain unconditioned reflexes it is necessary in studying the effect of thyroidectomy on the higher nervous functions of the sheep and goat to determine the influence of the athyroid condition on these unconditioned responses.

2. The incentives to maze learning are the gregarious instinct and the reaction to food. The gregarious instinct is not demonstrably weakened by thyroidectomy but the reaction to the presence of food, which may be regarded as a natural conditioned response, is inconstant following thyroidectomy.

3. Because of the persistent muscular weakness and recurrent periods of muscular prostration of the thyroidectomized animal the maze learning method has been supplemented by the motor conditioned reflex method of Bekhterev which does not depend upon locomotion.

4. The unconditioned reflex of Bekhterev's method is the defensive reaction of the foreleg to electrical stimulation. This unconditioned reflex persists even in the moribund cretin after the conditioned reflex to tactile stimulation has almost vanished.

5. It is suggested that diffuse inhibition may account for the lethargic and unstable reactions of the thyroidectomized sheep and goat.

I wish to acknowledge my indebtedness to Miss E. D. Simpson, Miss F. H. Burns and Dr. H. B. Sutton, for assistance rendered in this investigation.

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STEREOSCOPIC PROJECTION AND RIDGWAY'S STANDARDS AS METHODS OF INVESTIGATING COLOR BLINDNESS

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Stereoscopic projection affords an opportunity to make a distinction between conscious appreciation of color and the subconscious effect of color upon the retina. This note is intended to draw attention briefly to the possibility of a new method of attack upon the complex problem of color vision.

Stereoscopic projection has not yet come into general use for educational purposes though we have found it the most practical and important recent advance in technical methods of presentation.[1]

It has of course been employed sporadically in the cinema.

The principle is a very simple one. Stereoscopic lantern slides are projected through complementary red and green filters and the images superposed on the screen. The observer is provided with a spectacle frame, the lenses being simply plain glass filters, the one red and the other green. The observer's red eye picks out the red image and his green eye picks out the green image, each eye of course being oblivious of the image of the complementary color. Synthesis of the two images gives the illusion of perspective. The method is one which might well be used for other investigations for several reasons. The perspective may be altered at will. Vertigo and even vomiting can readily be induced through confusion of images. And lastly the observer actually has dissociated vision and accidents of imbalance are best avoided by sitting down to observe.

STEREOSCOPIC PERCEPTION IN THE COLOR-BLIND. During the last seven years we have had two color-blind students, both of the red-green type. Yet each of these students has perfect stereoscopic perception of the images on the screen in spite of his inability to identify the colors of the filters. Neither can identify ripe cherries or tomatoes without feeling the fruit. We have thoroughly tested the stereoscopic perception of both subjects of this experiment and, having failed to confuse either by turning the objects inside out (interchanging the lantern slides) and by flattening out perspective (insertion of identical slides in the double lantern) we have

[1] Harvey, H. F. Stereophotography and stereoprojection. 1922, International Medical Museums Bulletin, vol. viii, pp. 92–100.

assured ourselves of the normal sensation of perspective in both subjects. At the same time another experiment has been carried out on an individual with weak stereoscopic vision associated with slight external strabismus. This subject has gradually been educated to develop his stereoscopic perception but it is always easy to confuse him by the same manoeuvres which proved devoid of effect upon the color-blind.

It would appear therefore that the particular phases of color blindness evinced by our two subjects (H. D. I. and D. A. C.) are without effect so far as stereoscopic projection is concerned. This can mean only that the effect upon the retina of red and green complementary colors does not differ from the effect produced in normal persons, but that the actual appreciation of these particular colors is a function of the higher cerebral centres.

QUANTITATIVE ESTIMATION OF COLOR BLINDNESS. Having stated these particular facts it remains to define the degree of color blindness exhibited by each of our subjects. This is far from being an easy matter for color discrimination is readily modified by such simple factors as variations in attention and slight fatigue. Moreover color discrimination differs strikingly in individuals. The simplest example of this is given by asking a number of people to define the site where blue merges into violet. We have been compelled to abandon this particular question because of the great variation in response.

To define degrees of color blindness we have employed the following methods: perimeter, Stilling's charts, Jennings' wools, the spectroscope, color filters and Ridgway's color standards. Of all these methods the last two are the only ones which give a feeling of confidence in the result and since they are methods not hitherto employed we shall devote more attention to them than to the others.

Perimeter. Report for H. D. I. is the following.

Achromatic field: normal in size and shape in both eyes.

Blue field: both normal; slightly larger in left eye (80° temporal).

Yellow field: both normal; slightly larger in left eye (70° temporal).

Red field: limited in both eyes especially in the temporal direction.

The figures are: Right eye, 20, 15, 30, 18.
Left eye, 22, 22, 25, 18.

Green field: highly limited. No appreciation in either eye of the change from yellow to green except in a narrow approximately circular zone immediately surrounding the centre of the visual field. The light green disc of the standard perimeter appeared yellow beyond ten degrees in every direction.

Report for D. A. C. is the following.

Achromatic field: normal in size and shape in both eyes.

Blue field: both normal; slightly larger in right eye (80° temporal).

Yellow field: both normal; slightly larger in right eye (70° temporal).
Red field: limited in both eyes especially in the right.

The figures are: Right eye, 18, 15, 24, 20.
Left eye, 30, 20, 30, 25.

TABLE 1

Responses to Stilling's charts

REFERENCE TO CHART		IDENTIFICATION OF COLORED NUMBERS	
Group	Plate	H. D. I.	D. A. C.
I	1	Yes	Yes
I	2	Yes	With difficulty
II	1	No	No
II	2	No	Upper only
II	3	No	No
II	4	No	No
III	1	Yes	Yes
III	2	Yes	Yes
IV	1	No	No
IV	2	No	Lower only
V	1	Upper only	No
V	2	No	No
V	3	No	No
V	4	No	No
VI	1	No	No
VI	2	Upper only	No
VI	3	With difficulty	No
VI	4	No	No
VII	1	No	Upper only
VII	2	With difficulty	No
VIII	1	No	No
VIII	2	No	No
IX	1	Yes	Yes
IX	2	Yes	Yes
X	1	Yes	Yes
X	2	Yes	Yes
XI	1	Yes	Yes
XI	2	Yes	Yes
XII	1	Yes	Yes
XII	2	Yes	Yes

Green field: highly limited. No appreciation of green in either eye except in a restricted, approximately circular zone immediately surrounding the centre of the visual field, not extending beyond ten degrees in any direction.

We have taken the normal red and green fields as approximately the following:

Red field: 35, 30, 68, 45.
Green field: 20, 22, 22, 20.

Stilling's charts. The responses to Stilling's charts are given on the annexed table. Analysis of the replies appears to lead to no definite results.

Jennings' wools. At first sight this test might seem satisfactory in theory. But in nearly all colors in wools there is a percentage of neutral grey which, together with the inevitable soiling of standards and test wools, obscures the result. Both subjects carried out the test hesitatingly. It was necessary to make frequent comparisons between the standard and the yarn in question, showing that there was no definite mental picture of the colors red and green.

The following is the rough result.

H. D. I.

Green.

(1) Identified 4 out of 12 greens.
(2) Did not mistake any reds for greens.
(3) Called three colors green which were not green.

Red.

(1) Identified four out of eleven reds.
(2) Did not mistake any greens for reds.
(3) Called six colors red which were not red.

D. A. C.

Green.

(1) Identified six out of twelve greens.
(2) Did not mistake any reds for greens.
(3) Called fifteen colors green which were not green.

Red.

(1) Identified nine out of eleven reds.
(2) Did not mistake any greens for reds.
(3) Called fifteen colors red which were not red.

Obviously there is no lack of red-green discrimination by this test but there is failure to discriminate between greys, some blues and red on the one hand; and between browns and green on the other.

For a quantitative study of these results it was necessary to make an estimate of the percentage of red or of green in the various yarns. This can be done with approximate accuracy by reference to Ridgway's color standards (Ridgway, R., *Color Standards and Nomenclature,* Washington, 1912). From this volume one can obtain the relative amounts of each pure color, of white, black and neutral grey in the several yarns. With these figures to hand it is a simple matter to calculate the percentage of contained red or green. Reading the charts from above downward and from left to right we have recorded, in order, the percentages of pure red and green in the various red and green yarns and we have also indicated whether or not the color was identified (table 2). A careful study of the results

TABLE 2

Analysis of colors in Jennings' wools

COMMERCIAL NAME	COLORS PER CENT	TONE PER CENT	SHADE OF GREY PER CENT	FINAL PER CENT RED	IDENTIFIED	
					H. D. I.	D. A. C.
REDS:						
Venetian pink	Red 100	White 45 Color 55	Grey 32 Color 68	37.4	No	No
Pale rosolane purple	Red 74 Violet 26	White 22.5 Color 77.5	Grey 32 Color 68	40.0	Yes	Yes
Aster purple	Red 52 Violet 48	Black 45 Color 55	Grey 0 Color 100	28.6	No	Yes
Mallow purple	Red 52 Violet 48	White 9.5 Color 90.5	Grey 0 Color 100	47.1	Yes	Yes
Rosolane pink	Red 74 Violet 26	White 45 Color 55	Grey 32 Color 68	27.7	No	Yes
Cameo pink	Red 90 Violet 10	White 45 Color 55	Grey 32 Color 68	33.7	No	Yes
Amaranth purple	Red 74 Violet 26	Black 45 Color 55	Grey 0 Color 100	40.7	Yes	Yes
Rhodamine purple	Red 52 Violet 48	Color 100	Grey 0 Color 100	52.0	No	Yes
Thulite pink	Red 90 Violet 10	White 22 5 Color 77.5	Grey 32 Color 68	47 4	No	No
Pale rosolane purple	Red 74 Violet 26	White 22.5 Color 77.5	Grey 32 Color 68	40.0	No	Yes
Spinel pink	Red 90 Violet 10	White 9.5 Color 90.5	Grey 32 Color 68	55.4	Yes	Yes
STANDARDS:						
Mallow purple	Red 52 Violet 48	White 9.5 Color 90.5	Grey 0 Color 100	47.1		
Light paris green	Green 100	White 9.5 Color 90.5	Grey 32 Color 68	61.5		
GREENS:						
Scheele's green	Yellow 11 Green 89	Color 55 Black 45	Grey 0 Color 100	48.9	Yes	Yes

TABLE 2—*Concluded*

COMMERCIAL NAME	COLORS PER CENT	TONE PER CENT	SHADE OF GREY PER CENT	FINAL PER CENT RED	IDENTIFIED	
					H. D. I.	D. A. C.
GREENS cont.:						
Tiber green	Yellow 11 Green 89	White 22.5 Color 77.5	Grey 32 Color 68	69.0	Yes	No
Varley's green	Yellow 24 Green 76	Black 87.5 Color 12.5	Grey 32 Color 68	6.5	No	Yes
Viridian green	Green 93 Blue 7	Black 45 Color 55	Grey 0 Color 100	51.1	No	No
Light paris green	Green 100	White 9.5 Color 90.5	Grey 0 Color 100	61.5	Yes	Yes
Winter green	Yellow 11 Green 89	Black 45 Color 55	Grey 32 Color 68	33.3	No	Yes
Viridian green	Green 93 Blue 7	Black 45 Color 55	Grey 0 Color 100	51.1	No	No
Viridine yellow	Yellow 39 Green 61	White 9.5 Color 90.5	Grey 0 Color 100	55.2	No	No
Asphodel green	Yellow 39 Green 61	Color 100	Grey 77 Color 23	14.0	No	Yes
Vanderpoel's green	Yellow 11 Green 89	White 9.5 Color 90.5	Grey 0 Color 100	80.5	No	Yes
Oriental green	Yellow 11 Green 89	Color 100	Grey 0 Color 100	89.0	Yes	No
Neuvider green	Green 93 Blue 7	White 22.5 Color 77.5	Grey 0 Color 100	72.1	No	No

convinces us that they lead nowhere and that the method itself is unsatisfactory in consequence of fatigue and soiling of the wools. We believe that a more useful result is obtained by the direct use of Ridgway's standards themselves.

The spectroscope. This instrument was used in the manner recommended by G. J. Burch (Cases of color blindness together with eleven selected examples of normal color sensations. Phil. Trans. Roy. Soc., B. 1908, cxcix, pp. 231–251). We found it a very slow, tedious and technically difficult method, calculated to fatigue the subject before proper

records could be taken. The standardization of the instrument need not be recorded: suffice it to state that the normal control (T. W. T.) found the red-yellow mergence at 10.0 on the arbitrary scale the yellow-green at 12.0 and the green-blue between 32.0 and 33.0: he could not identify the position of the blue-violet. One subject (H. D. I.) thought he could identify the red-yellow mergence at 5.0, could not find at all the yellow-green, and placed the green-blue at 32.0. The other subject (D. A. C.) found the red-yellow mergence at 8.0, placed the yellow-green at 31.0 and the green-blue at 32.0. It is apparent then that the defect in color vision in both students lies solely in the red-yellow-green part of the spectrum: the green-blue-violet vision is normal.

From the other preceding investigations it appears that both men can identify pure red and pure green though they can do this only with difficulty and no very clear memory of the color impression is formed. This fact comes out more strikingly still when they are asked to identify border zones and mixtures of colors. Their condition seems an exaggeration, in another part of the spectrum, of the uncertainty of the normal person regarding the blue-violet mergence.

Color filters. Having reached this stage in the investigation we returned to the projection experiments but this time confined ourselves to the projection of color alone. Both subjects identified the red field at once. When the green field was projected both reported it white. They were unable to distinguish the difference in color when the red field was superposed upon the green, a manoeuvre which results in the formation of a really white field on the screen. When the red and green fields were permitted to overlap on the screen both students could identify the red and green fringes though they called the latter blue. Hence, in juxtaposition, both could discriminate between white and green light but neither is able to form a memory picture vivid enough to enable him to differentiate between white and green when exhibited separately. This bears out the Jennings test.

Both students have a sensation of blue when the green field is observed through the green spectacle glass. When the green field is examined through the red glass it appears much reduced in intensity. When the red field is examined through the green glass it is just a duller red. These are normal reactions.

The method of color filters permits a rapid examination without fatigue.

Ridgway's standards. By the method of Ridgway's standard colors we obtained rapidly by far the most exact and definite response. This was the only experiment which resulted in prompt and unhesitating replies. The record is the following.

H. D. I. Normal discrimination between red and yellow. No distinction noted between Y (100 per cent yellow) and YG-Y (75 per cent

yellow and 25 per cent green) nor indeed is there any color discrimination in following the series of yellow green mixtures. But when emerald green (100 per cent green) is reached there is a sudden increase in intensity. Up to this point the admixture of green merely darkens the yellow. Emerald green is not recognized as a definite color in the manner in which blue is recognized. It gives a sensation of heightened intensity and is called "buff." The first admixtures of blue with green again are drab and meaningless: they are not definite colors like yellow and blue. But when the stage BB-G (75 per cent green, 25 per cent blue) is reached blue is definitely and suddenly recognized. Blues beyond this are definite though violets are reported as merely deeper blues. This last reaction is not uncommon among people who are supposed to have normal color vision. In the red-violet mixtures true purple, RR-V (33 per cent red, 67 per cent violet) appears as blue alone. It is not until the stage RV-R (74 per cent red, 26 per cent violet) is reached that the red component is recognized.

D. A. C. The distinction between red and yellow is recognized but the admixture of green with yellow merely darkens the yellow until the stage GG-Y (39 per cent yellow, 61 per cent green) is reached. At this point green is first registered. Changes in the green mixtures are then recognized up to the stage BB-G (75 per cent green, 25 per cent blue) when blue first makes a definite sensation. It is a little difficult to decide whether or not the uncertainty regarding the green-blue mergence is simply confusion of learning as in the blue-violet change, but this is not really important if our view be sustained that color blindness is an exaggerated form of what appears at the blue-violet junction in otherwise normal persons. Further admixtures of blue are discriminated and violets are recognized as dark blue. In the red-violet mixtures true purple, RR-V (33 per cent red, 67 per cent violet) appears as blue only. Red is first recognized in Tyrian rose, RV-R (74 per cent red, 26 per cent violet) but it is also discriminated in Rood's violet for which the formula is: red 33 per cent, violet 67 per cent; black 45 per cent, color 55 per cent. It is also noted in aster purple, again a dark tone, namely, red 52 per cent, violet 48 per cent, black 45 per cent, color 55 per cent.

At first we thought that possibly the admixture of black or even of grey to form a darker tone or shade might be a factor in the erratic responses to Jennings' yarns, but a little figuring with a pencil on table 2 left the situation just as obscure as before. We must be content to leave the responses to Jennings' test without explanation. There are influences at work in color vision which continue to elude all search.

In conclusion we would acknowledge our indebtedness to Dr. George B. Ray for the instruction and help he generously gave us in setting up and calibrating the spectroscope.

SUMMARY

1. The evidence gathered from the records of the two red-green color blind subjects investigated in this work shows that, in spite of inability to discriminate greens, there is no abnormal effect upon the retina and therefore the color-blind person has the typical illusion of perspective in stereoscopic projection. This seems to indicate that color perception is a function of the higher cerebral centres rather than of the retina.

2. Analysis of the percentage of red or of green in the several yarns of of Jennings' test brings to light no ordered response. We incline to regard the erratic character of the result as partly, at least, due to fatigue.

3. The method of Ridgway's standards affords an easy, rapid and non-fatiguing means of expressing quantitatively the degree of color blindness.

4. The two cases under discussion, while differing from each other in degree, are alike in that they exhibit extreme difficulty or even inability to memorize green, and show their defect in the yellow-green mixtures rather than in the pure colors themselves.

STUDIES ON THE PHYSIOLOGY OF SLEEP

IV. The Effect of Sleep on Respiration

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All investigators agree that there is a change in the rate and character of the respiratory movements with the onset of and during sleep. As in the case of cardiac activity, some of these changes may be connected with the assumption of the horizontal position preparatory to going to sleep. Piéron (1913), on the basis of twenty determinations, found that the average respiratory rate was 17 per minute with the subject standing, and that this rate fell to 12 merely as a result of lying down. But certain respiratory phenomena have been observed to be directly dependent upon passing from the waking to the sleeping state, and a similar set of events, but in the reverse order, was observed upon awakening. Broadbent (1877) described something like Cheyne-Stokes breathing in a normal man 80 years of age, during sleep. Mosso (1878), working on five individuals, noticed a certain periodicity in the respiratory movements during sleep in only one subject, and he called it Cheyne-Stokes breathing, even though his records fail to show any periods of actual apnea. According to Bechterew (1910) the dorsal decubitus position is often sufficient to cause the appearance of a periodicity in respiration. Mosso in the same paper reported an inversion of the respiratory type, i.e., the predominance of thoracic over abdominal breathing during sleep, the opposite relationship prevailing in the waking state. Bechterew (1910) ascribes this phenomenon to the subject's lying on his back, as he saw the respiration resume its abdominal character when the subject changed from dorsal to lateral decubitus. Shepard (1914), working on two subjects, noticed that the changes in respiration brought about by sleep were not the same in his numerous experiments, and he described three different types of breathing during sleep. He, too, observed that there was a decrease in the amplitude of the abdominal movements and a relative increase in chest breathing, and he considered this the most characteristic change in connection with the oncoming of sleep.

Some time ago each of us had independently observed a respiratory

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periodicity during the onset of sleep, and in this study it was our purpose to reinvestigate the respiratory phenomena said to occur during sleep, and especially the events that accompany the passing from the state of wakefulness to sleep, and vice versa.

Method. The experimental procedures employed in this investigation were relatively simple. Two adjoining rooms in an isolated situation were equipped as follows: In the sleeping room, next to the wall separating it from the experimenter's room, was placed a comfortable cot. Attached to the wall about 2 feet above the head of the cot was a rather loud telegraphic clicker operated by the observer in the outer room. In this outer room, close to the wall that separated it from the subject's room, was an extension kymograph operated at a slow speed, approximately 1 inch per minute. One signal magnet was placed in circuit with a spring key and with the telegraphic clicker, in order to record the auditory stimuli sent by the observer into the subject's room; a second signal magnet connected with a pear-shaped push-button key which the subject held in his hand served to record the response to the stimulus, as the subject was instructed to press the button immediately upon hearing the sound of the telegraphic clicker. The other recording apparatus consisted of two tambours and a time-marker. All writing points were on the same ordinate, when the tambours were deflated. The tambours were connected by extra heavy tubing to two sensitive pneumographs placed, one high around the subject's chest, the other around the abdomen at about the level of the umbilicus. The tubes leading from the pneumographs to the tambours as well as the wires connecting the signal keys with the magnets were passed through a small hole in the wall.

After the subject had been placed on the cot and the pneumographs adjusted, the light was turned out and the door to the subject's room closed. He was thus left completely alone and in the dark. The door was not opened during the experiments, except in a few cases, when the subjects were deeply asleep and failed to respond to repeated stimuli. In such cases the operator entered the subject's room to make some additional observations or to awaken the subject, as the occasion demanded.

Nine healthy adult subjects, ranging in age from 20 to 40 years, were employed. Two of these were women. Selection was made of subjects that experienced difficulty in sleeping at odd times and under unusual circumstances as well as of persons who could readily sleep under any and all conditions. Most experiments were performed early in the afternoon shortly after the subjects had their lunch, but in a few instances the tests were made late in the afternoon or at night (around 11 o'clock). No experiment lasted more than 2½ hours. In some experiments the subjects were asked to assume the dorsal decubitus position, in others the lateral decubitus, and only rarely did they change their positions while asleep

A total of thirty experiments was included in this study. With one exception, at least two tests were made on each subject, so that there are observations with each one lying on the back and on one side. Since it was not found practical to get the same degree of pressure in both pneumographs, the amplitude of the excursion of each tambour as registered on the pneumograph record was measured and the alterations in the ratio between the thoracic and abdominal excursions noted.

An attempt was made to observe three phases or periods in the condition of the subject: first, a period of wakefulness, both before going to sleep and after waking, with the lights on and the door open; second, a transitional period representing the process of going to sleep which we shall designate for convenience as somnolence, a period when the subject is semiconscious, not widely awake nor completely asleep; third, a period during which the subject is definitely asleep. During the first and second periods, which were of variable duration, the subjects responded to the signals promptly or with very little delay. During the third period there was usually either a definitely delayed response (five seconds or more), or the subject failed to respond altogether. Both of the latter were considered as criteria of sleep. In addition, however, evidence was obtained by direct inspection of the subject by the observer and by hearing certain of the subjects snore.

Results. So far as could be determined, neither the time of day, nor the age, nor the sex of the subjects had any influence on the nature of the changes that occurred during somnolence and sleep. The normal respiratory rate while lying down and awake varied in different subjects from 8 to 25 per minute, the average being 18. The changes in rate during somnolence were manifold. In sleep, in 5 of the 30 experiments the rate was definitely slowed, in 12 there was no change, and in 13 it was definitely increased. In 18 experiments there was a return to approximately the original rate on waking. The normal waking rate varied in the same individual in successive experiments, as did also the type of change in somnolence and after awakening. It was not possible to correlate these variations in the same individual with any other recognizable factor.

The changes occurring in the regularity of respiration were most striking, although here again there was no constancy of results. In 16 of the 30 experiments the respiration showed a distinct irregularity in both the thoracic and abdominal movements during the period of somnolence. In only one subject was the respiration regular in every experiment (fig. 1). No subjective or objective phenomena were observed which would account for the changes in the character of respiration, and in successive tests on the same subject the irregularities were not always the same. As a general rule, if the respiration was regular in the beginning of the experiment, it became very irregular during somnolence, assuming a regular sequence

Fig. 1

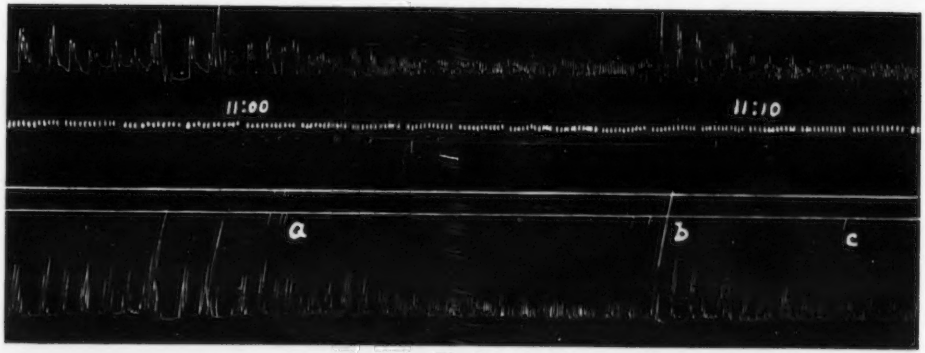

Fig. 2

Fig. 1. Subject IV, male. June 3, 1925, 12:45 p.m. Did not sleep the night before. Dorsal decubitus. Tracings from below up, abdominal respiration, signal of observer, response of subject, time in 5 seconds and in minutes, thoracic respiration. At *a*, 2 minutes after light had been turned off, the subject readily responded to signal; 10 minutes later subject was fast asleep, failed to respond to three stimuli, and reponded to the fourth, as shown at *b*. Practically no irregularity at any time.

Fig. 2. Subject IX, male. July 6, 1925, 10:35 p.m. Dorsal decubitus. Tracings from below up, abdominal respiration, signal of observer, response of subject, time in 5 seconds and in minutes, thoracic respiration. At *a*, 25 minutes after the beginning of the test, subject responded tardily to one stimulus and promptly to two stimuli closely following. Subject in state of somnolence, respiration periodic. At *b*, 5 minutes later, subject asleep, respiration regular, failed to respond to two stimuli, responded to third, returned to the state of somnolence. At *c* subject is asleep again, respiration regular, does not respond to stimulus.

Fig. 3

Fig. 3. Subject III, female. July 17, 1925, 10 p.m. Lateral (right) decubitus. Subject tired and sleepy. Tracings from below up, signal of observer, response of subject, abdominal respiration, time in 5 seconds and in minutes, thoracic respiration. From the start periodicity apparent only in thoracic breathing. At *a* subject responded to stimulus, but shortly afterward she was asleep, thoracic respiration becoming regular. At *b* she failed to respond to the first two stimuli, but responded to the third, remaining awake, as shown by the reappearance of the irregularity in the thoracic breathing and the response to stimuli at *c*.

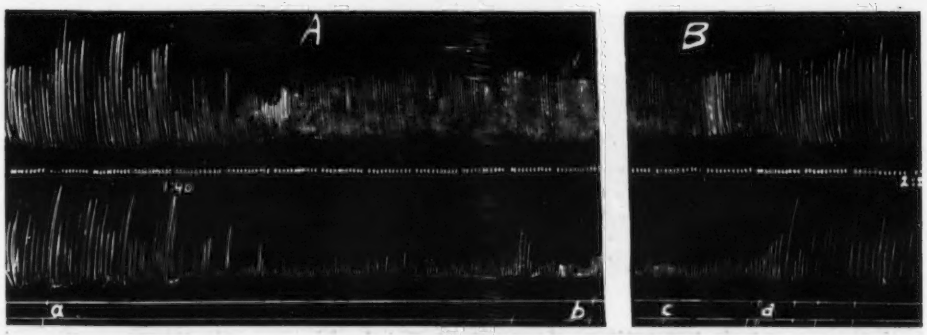

Fig. 4

Fig. 4. Subject I, male. August 11, 1925, 1:30 p.m. Dorsal decubitus. Tracings from below up, signal of observer, response of subject, abdominal respiration, time in 5 seconds and in minutes, thoracic respiration. At *a* the subject is awake, responds to stimuli, the respiration is irregular in extent and rate. As the subject falls asleep, the respiration becomes more regular, shallower and faster. At *b* the subject is asleep, fails to respond to the first, but responds to the second stimulus. At *c* the subject is still asleep, at *d* he is awakened and kept awake by repeated stimuli; the respiration again becomes irregular.

again in sound sleep. On waking it sometimes became irregular at first, changing to regular after some time (fig. 2); in other cases it continued regular upon awaking.

In some instances the irregularity was so pronounced and so definitely periodic in character as to resemble Cheyne-Stokes breathing. In most cases the irregularities were synchronous in both the abdominal and thoracic movements, but in a few experiments marked periodicity appeared in one of these two types of respiratory movements without being apparent in the other (fig. 3). Usually the irregularity took the form of a considerable shortening of the amplitude of the excursions as compared to normal, lasting for two or three respiratory cycles. In a few experiments, however, there was, instead, at varying intervals a deep inspiration, which we shall call a "ruminating sigh," since a similar phenomenon is always observed in ruminating cattle. In about half of the experiments the variations in amplitude of whatever type were distributed evenly enough to be designated as periodic, but the irregularity was often periodic and arhythmic at different times in the course of the same experiment. Very frequently during sleep the sound of the clicker brought about some modification of respiration although the subject was too deeply asleep to respond. The type of modification was not constant, but it usually expressed itself in a deep sigh-like inspiration.

One subject, a young student, maintained a regular respiration throughout the experiment, with practically no modifications of any kind at any time (fig. 1). He responded to the signal, after some delay, by pressing the key, even when direct observation proved him to be sound asleep. This very deep sleep was at first thought to have been associated with the fact that he had not slept at all the night before, but a later experiment, the subject having had $8\frac{1}{2}$ hours of sleep the night before, gave similar results.

In studying the amplitudes of the abdominal and thoracic excursions and their relation to each other, the actual extent of these movements as registered on the kymographic record was measured and the ratio between the two utilized to determine the relative changes that occurred in these two types of respiratory movements. For convenience this relationship is expressed as the T:A ratio. In 17 experiments this ratio was so altered as to indicate a decreased depth of the abdominal excursions. This decrease was absolute in 15 experiments. The thoracic excursion was increased absolutely in 13 experiments, was decreased in 10, and remained unchanged in 7 (figs. 4 and 5). Actual inversion of the T:A ratio did not occur in a single experiment. In two experiments there was no change in the ratio, both the thoracic and the abdominal movements decreasing in the same proportion as a result of sleep.

Like Mosso, we noticed that the thoracic inspiration begins before the

abdominal, the difference becoming slightly more marked in sleep than in wakefulness.

In all our experiments the position of the subject, whether dorsal or lateral decubitus, did not affect the results in any way.

Discussion. The results obtained in the 30 experiments on 9 subjects are characterized by a complete lack of uniformity insofar as respiration during wakefulness and somnolence is concerned. The only constant finding was the relatively regular and even breathing observed during sleep. The common belief that respiration is slowed in sleep could not be substantiated, as only in 5 experiments was the rate definitely slowed. In the others there was either no change or the rate was increased as a result of sleep. Like Piéron, we observed in some cases a slowing, instead of an accelerating of respiration, following awakening.

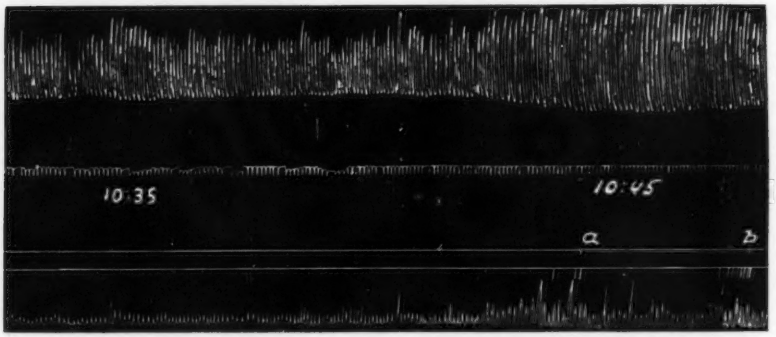

Fig. 5. Subject VI, female. July 20, 1925, 10:30 p.m. Dorsal decubitus. Tracings from below up, abdominal respiration, signal of observer, response of subject, time in 5 seconds and in minutes, thoracic respiration. As the subject fell asleep, both the thoracic and abdominal respiration became deeper. Rate unchanged or slightly increased. At *a* and *b* responses are obtained only after repeated stimulation.

As regards irregularity of respiration, periodic or aperiodic, we found evidence of it in some subjects in the waking state (fig. 4), and in about half of all our experiments during somnolence. In some cases there was a periodic irregularity resembling Cheyne-Stokes breathing, and strangely enough, it appeared at times only in the thoracic or only in the abdominal respiration. It would seem that the centers governing the contractions of the diaphragm and those of the intercostals have each a different irritability, but then it is hard to understand how the center having the lower irritability ever is stimulated, since the activity of the other center prevents the accumulation of CO_2 in the blood. Perhaps in this case it is a question of summation of nervous rather than chemical stimuli. The fact that the

thoracic inspiration begins before the abdominal would also tend to support the idea of a difference in the irritability of the two motor respiratory centers. Bechterew's suggestion that the periodicity is dependent upon the dorsal decubitus position is not borne out by our experiments. Indeed, in some subjects we observed periodic breathing in lateral, and not in dorsal decubitus.

When the irregularity of the respiration disappeared, it was almost always an evidence of the subject's being soundly asleep. A loud auditory stimulus may temporarily disturb this regular breathing, but unless the subject awakens and remains awake, the respiration soon again becomes regular (fig. 2). As said above, that is our one constant finding in this study. Unfortunately, our tests were all of relatively short duration, and we cannot tell whether the respiration would become irregular, were we able to carry the experiments through a whole night's sleep. It is possible that various noises and other stimuli (even internal and therefore unrecognized by the observer) more than once disturb the regularity of respiration during a night's sleep.

The decrease in the amplitude of the abdominal movements and the increase in chest, the so-called inversion, described by Mosso and considered by Shepard the most characteristic respiratory phenomenon in sleep, appears from our results not to be an invariable accompaniment of that state. In some instances we got an increase in the thoracic movements, in others a decrease, in still others no change. The same may be said of the abdominal movements. A relative inversion, characterized by an increase in the T:A ratio was observed in only about half of our experiments. Thus we are unable to agree with Shepard on its being characteristic of sleep.

In general, on the nine subjects we employed we found such a variety of changes concomitant with wakefulness, somnolence, or sleep, as to conclude that there is no direct relationship between these varied states and the rate and regularity of respiration. Since respiration is very easily affected by all kinds of afferent impulses reaching the central nervous system, all the changes that occur during wakefulness and somnolence may well be due to greater or lesser relaxation of the body musculature on lying down (number of proprioceptive impulses), and to the abundance or poverty of mental processes in these two periods. The relative regularity of respiration in sleep is probably the result of complete muscular relaxation and of the failure of other afferent impulses to reach the respiratory center. The classical notion of very marked and constant changes in respiration symptomatic of sleep should, in our opinion, be abandoned.

SUMMARY

1. The respiratory rate was frequently increased on passing from wakefulness to sound sleep, although in nearly as many cases there was no change in rate. Very slow normal rates were always increased.

2. If the respiration was even and regular while awake, it usually became progressively less regular during somnolence, but became regular again in sound sleep. If respiration was irregular in the beginning, this irregularity usually persisted throughout somnolence, sometimes becoming more pronounced, but generally disappearing during deep sleep.

3. The irregularity in respiration was commonly aperiodic, but it sometimes assumed a definite periodicity, resembling Cheyne-Stokes breathing. While changes in the thoracic and abdominal movements were ordinarily the same, pronounced irregularity sometimes occurred in one of these movements alone.

4. The so-called inversion of the ratio between the thoracic and abdominal amplitudes on going to sleep did not take place. In the majority of experiments the amplitude of the abdominal excursions was absolutely decreased on going to sleep, and in a number of experiments the amplitude of the thoracic excursions was absolutely increased.

5. The position occupied by the subject during the experiment did not seem to influence the direction of the changes occurring.

6. A subminimal auditory stimulus that failed to elicit a response from the subject was frequently sufficient to produce some modification of respiration.

7. The changes observed were not always the same in a given subject in successive experiments, nor even in the same test on being awakened and allowed to fall asleep again.

We wish to thank our colleagues who kindly agreed to serve as subjects for this study.

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THE ACTION OF INSULIN ON THE DOMESTIC FOWL

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It has been shown by Bierry and Fandard (1912) that the blood sugar of the fowl is about double that of mammals, ranging from 180 mgm. to 200 mgm. per 100 cc. It was of interest, therefore, to ascertain the extent to which insulin would lower the blood sugar of the fowl, and if possible to determine the effects of severe hypoglycemia. In a previous communication the authors (1925) pointed out that the function of the heat-regulating mechanism in dogs and cats is impaired or completely inhibited by hypoglycemia.

Method. Domestic fowls of three varieties were used—white Leghorns, Plymouth rocks and Rhode Island reds. The birds were kept on a diet of crushed corn and oats. Some were fed until the time of use; others were starved for various periods. Insulin was injected under the skin of the thigh. Deep rectal temperature was recorded, the bulb of the thermometer being inserted to a depth of 6 cm. to 8 cm. Blood for sugar determinations by the method of Folin and Wu was obtained in 1 cc. quantities from the wing vein. The body temperature of the animals was lowered by immersing them in tap water.

The blood sugar of eleven normal fowls has been determined, and has been found to vary between 161 mgm. and 260 mgm., the average being 200 mgm. per cent (table 1).

In order to ascertain whether reducing substances other than glucose are in part responsible for these high values, 1 cc. quantities of blood were diluted to 8 cc. with a suspension of yeast in distilled water. This mixture was kept at room temperature for 24 hours, and Folin and Wu determinations were carried out. A control was run on a 0.2 per cent glucose solution. The results are shown in table 2. The conclusion can be drawn that in hen's blood the greater part of the material responsible for the reduction obtained by the blood sugar method of Folin and Wu is glucose, but that about 15 per cent of the reduction is due to substances not fermentable by yeast.

Three well-fed birds received insulin in doses of 4 units per kilo. Blood

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samples were taken at 20-minute intervals in order to trace the fall of blood sugar and its subsequent return to normal (figs. 1, 2, 3). The curves so obtained differ from those of mammals. For purposes of description the insulin curve of birds may be divided into three parts: 1, preliminary fall; 2, secondary fall; 3, recovery. While no preliminary rise

TABLE 1

	REMARKS	NORMAL BLOOD SUGAR	INSULIN INJECTIONS	MINIMUM BLOOD SUGAR REACHED	
		mgm. per cent	*Units per kilo*	*mgm. per cent*	*hours after insulin*
Cock 7	Well fed	188	4	115	3
Cock 12	Well fed	212	4	125	3
Cock 14	Well fed	188	4	111	1
Cock 2	Well fed	220	25	101	3
Cock 1	Well fed	196	50	74	2
Cock 3	Well fed	260	35	142	2
Cock 2	Well fed	237	130	167	3
Cock 1	Well fed	161	10 per hour for 5 injections	55	2
Hen 4	Well fed	197	10 per 2 hours for 3 injections	75	8
Hen 5	Well fed	230	10 per 2 hours for 3 injections	105	8
Hen 6	Well fed	216	10 per 2 hours for 3 injections	57	9
Cock 7	Well fed	167	10 per 2 hours for 3 injections	101	8
Hen 10	Well fed	184	15 per hour for 6 injections	55	3
Cock 13	Starved 12 days	181	4	96	2
Hen 10	Starved 44 hours	186	10 per hour for 6 injections	62	4
Hen 4	Starved 72 hours	186	10 per hour for 6 injections	79	3
Cock 11	Starved 144 hours	178	15 per hour for 5 injections	77	4

TABLE 2

	SUGAR CONCENTRATION BEFORE FERMENTATION	SUGAR CONCENTRATION AFTER FERMENTATION
	mgm. per cent	*mgm. per cent*
Hen 6	224	30
Cock 12	230	36
Glucose solution	218	(14?)

was ever observed, in two animals the fall of blood sugar during the first twenty minutes was relatively slight (figs. 1 and 2). The preliminary fall is very rapid and extensive, the blood sugar concentration decreasing by about 70 mgm., as compared with 40 mgm. in mammals. Its duration is about the same as in mammals—from 40 minutes to 1 hour. The

secondary fall, if it may be so termed, is characterized by rather extensive and irregular oscillations. The recovery commences between 3 and $6\frac{1}{2}$ hours after the administration of insulin, and the blood sugar is again normal some hours later. The lowest blood sugar level observed in any of these animals was 111 mgm. per cent. The symptoms characteristic of hypoglycemia in mammals were not observed.

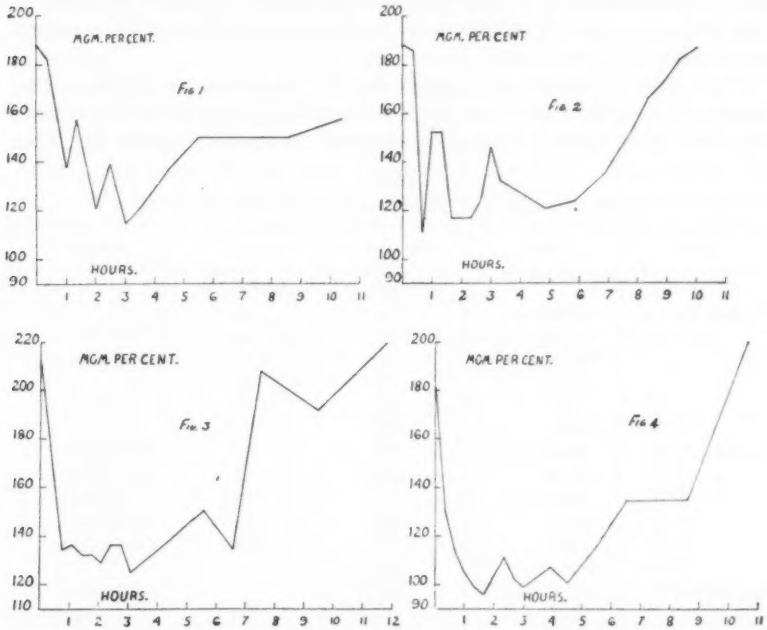

Fig. 1. Insulin curve of a well-fed fowl (cock 7). Dose, 4 units per kilo.
Fig. 2. Insulin curve of a well-fed fowl (cock 14). Dose, 4 units per kilo.
Fig. 3. Insulin curve of a well-fed fowl (cock 12). Dose, 4 units per kilo.
Fig. 4. Insulin curve of a fowl (cock 13) after 12 days starvation. Dose, 4 units per kilo.

In order to produce more profound hypoglycemia, larger doses of insulin were given. Amounts ranging from 10 to 130 units per kilo were injected, but the minimum level obtained under these conditions was 55 mgm. (table 1). Even after these large doses the blood sugar returned to normal.

It was thought possible that excretion of insulin might have been responsible for the failure of the blood sugar to fall below 55 mgm. per cent. Six birds, therefore, received several successive 10 or 15 unit doses of

insulin at intervals of 1 or 2 hours. The minimum blood sugar obtained as a result of this procedure was again 55 mgm. (table 1).

The fact that the blood sugar did not decrease to a greater extent might have been due to rich glycogen stores. In order to deplete this source of glucose several birds were starved for periods ranging from 44 hours to 12 days. The behavior of the blood sugar in a starved animal after administration of 4 units of insulin per kilo is illustrated in figure 4. The curve corresponds very closely to that of a well-fed bird, even as regards complete recovery.

Three starved birds were given hourly injections of insulin. The lowest concentration to which the blood sugar fell was 62 mgm. per cent. One fowl after 7 days' starvation received 50 units of insulin per kilo.

TABLE 3

Cock 11; weight 2.92 kilos. Starved since September 15

DATE	TIME	BLOOD SUGAR	INSULIN	REMARKS
		mgm. per cent.	*units per kilo*	
September 21	10:00 a.m.	178	15	
	11:40	120	15	
	12:40 p.m.	81	15	Bird active
	2:40	77	15	
	3:40	91	15	
	5:00	109		
September 22	10:50 a.m.	90	15	Bird quiet
	11:50	100	15	Bird quiet
	12:50 p.m.	71	15	Bird quiet
	2:50	60	15	Bird active
	4:30	72	30	Bird active
	6:00	50		
September 23	10:20 a.m.	55		Bird quiet
	12:20 p.m.	77		Bird restless
	5:00	57		Bird quiet

The blood sugar returned to normal in less than 48 hours. In a final attempt to produce a more severe hypoglycemia, three birds after starvation were given hourly doses of insulin on two or three days in succession. The effect of this procedure upon the blood sugar in one of these animals is shown in table 3. The lowest blood sugar level reached on the first day was 77 mgm., and on the second day 50 mgm. per cent. In spite of the fact that this bird received no insulin on the third day, the hypoglycemia persisted. In another animal the minimum was 37 mgm. per cent, this level being reached on the second day. From these experiments the conclusion can be drawn that starvation does not appreciably affect the extent to which insulin lowers blood sugar in the fowl.

No convulsions, tremors, or muscular twitchings were observed in any

of the hypoglycemic animals. However, when the blood sugar reached about 100 mgm., the birds became disinclined to move and, as the blood sugar level decreased, weakness and unsteadiness became more and more apparent. In some cases the birds were unable to stand or to hold up their heads. These symptoms gradually disappeared as the blood sugar rose. A constant effect of hypoglycemia is a fall in body temperature of from 1° to 3°C. (table 4). This decrease in temperature does not begin until the blood sugar reaches a level of between 140 mgm. and 100 mgm. per cent. As the blood sugar continues to decrease there is a concurrent diminution in body temperature, but a subsequent rise of blood sugar is almost immediately followed by a rise in body temperature. Shivering never accompanies this reduction of body temperature.

The normal fowl shivers when immersed in water at a temperature of 16°C. The onset of shivering may occur without change in rectal temperature, or may be preceded by a fall of 0.3° to 0.8°C. It has been

TABLE 4

Hen 4; weight 2.82 kilos

TIME	INSULIN	BLOOD SUGAR	TEMPERATURE
	units per kilo	*mgm. per cent*	°C.
10:00	10	186	41.5
11:00	10	110	41.5
12:00	10	86	41.0
1:00	10	79	40.0
2:00	10	81	40.0
4:00	10	90	40.5
5:00	10	94	41.0
6:00	10	96	41.0

pointed out by the authors (1925) that shivering is abolished in mammals by hypoglycemia. The following experiments were undertaken to determine whether this likewise applies to birds. The blood sugar of a hen was lowered to 63 mgm. per cent by means of insulin. The body temperature at this time was 40.8°C. The animal was then immersed in tap water (20°C.). Shivering began only after 25 minutes had elapsed, at which time the animal's temperature was 33°C. A blood sample was taken as soon as shivering began, and it was found that the sugar concentration had risen to 107 mgm. per cent. This experiment was repeated twice as shown in table 5. In the case of hen 10a, shivering was extremely slight when first observed, and the blood sugar concentration had not risen during the 4 minutes of immersion. After 16 minutes' further exposure to cold the blood sugar had risen to 58 mgm. per cent, and shivering had become violent. These experiments indicate that shivering in fowls is inhibited by hypoglycemia, and that these animals

raise their blood sugar levels autogenously when they are immersed in cold water in a hypoglycemic condition.

Several experiments were carried out to determine the effects of cooling alone on these animals. Merely keeping a thoroughly wet bird in a cold air chamber (8°C.) is not sufficient to lower its body temperature more than 2°C. in 3 hours. On the other hand, by immersion in tap water (20°C.) for an hour the body temperature can be lowered as much as 15°C. By this means one fowl was cooled to 22.5°C., at which temperature death occurred. This animal shivered vigorously and remained fully conscious until a few minutes before death, which was preceded by collapse. Another bird was cooled to 28°C., then removed from the

TABLE 5

	BLOOD SUGAR BEFORE IMMERSION	TEMPERATURE BEFORE IMMERSION	TIME BETWEEN IMMERSION AND SHIVERING	BLOOD SUGAR WHEN SHIVERING BEGAN	TEMPERATURE WHEN SHIVERING BEGAN
	mgm. per cent	*°C.*	*minutes*	*mgm. per cent*	*°C.*
Hen 4	44	39.7	15	63	37.0
Hen 10	62	40.8	25	107	33.0
Hen 10a	48	41.0	4	48	38.0

TABLE 6

	NORMAL		AFTER COOLING	
	Blood sugar	Temperature	Blood sugar	Temperature
	mgm. per cent	*°C.*	*mgm. per cent*	*°C.*
Hen 8	216	42.0	208	22.5
Hen 9	212	42.6	204	29.0
Hen 4	176	42.0	140	31.5
Hen 6	192	41.5	172	29.3

bath, and placed in the sunshine. It shivered vigorously, and recovered its normal temperature in 4 hours.

After the body temperature of two other birds had been lowered, insulin was injected to determine whether hypoglycemia would abolish or retard the return of the temperature to normal. In both cases recovery was effected in about 6 hours. A third bird injected with insulin before cooling behaved in a similar manner. These experiments indicate that recovery of normal temperature is somewhat retarded by hypoglycemia; but there is no tendency for the temperature to remain at a low level, nor do the birds lose consciousness at any time during the experiment.

The blood sugar was determined in several fowls before and after lowering their body temperature. In every case it was found that cool-

ing had produced a diminution in blood sugar concentration (table 6). Insulin reduces blood sugar to approximately the same extent in the cooled as in the normal bird.

CONCLUSIONS

1. The blood of normal fowls contains about 200 mgm. per cent of reducing substances estimated as glucose by the blood sugar method of Folin and Wu. Of this, 15 per cent is not fermentable by yeast.

2. Although the extent of the fall of blood sugar following insulin administration in fowls is greater than in mammals, convulsions do not occur. Single doses of insulin, whether of 4 or of 130 units per kilo, produce approximately the same amount of reduction of blood sugar. Repeated injections, however, have a greater effect. Even after massive doses of insulin the blood sugar of fowls returns to normal autogenously. The severity of the hypoglycemia following insulin injection in fowls is not increased by previous starvation.

3. Lowering of the blood sugar concentration by means of insulin in fowls causes a fall in body temperature which is not accompanied by shivering.

4. When fowls with profound hypoglycemia are immersed in cold water, they do not shiver at once. Their blood sugar concentration soon rises, however, and this is followed by shivering.

5. Fowls do not lose consciousness when cooled to 27.5°C., and are able to recover their normal temperature spontaneously. Hypoglycemia retards this recovery.

6. Lowering of the body temperature of fowls produces a diminution in the concentration of the blood sugar.

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STUDIES ON THE PHYSIOLOGICAL ACTION OF LIGHT

VI. Alterations in Cell Electrophoretic Potential Produced by Direct Irradiation of the Blood in Vivo

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In preceding papers of this series (Reed, 1925b) have been described the technic and results of direct irradiation of blood circulating through a quartz tube inserted in an artery in dogs under ether anesthesia. It is the purpose of this paper to report briefly the results of some observations on changes in electrophoretic potential of the red blood cells resulting from irradiation.

The measurements of electrophoretic potential were made on cells suspended in 1:100–1:120 dilution of the blood in 0.85 per cent NaCl solution. The details of the method, apparatus and calculations have been reported elsewhere (Falk, Gussin and Jacobson, 1925).

In the following table are given the results of electrophoretic potential determinations on the red blood cells of dogs subject to ether anesthesia. In all cases the blood samples were heparinized (Reed, 1925a). An average of one and one-half hours elapsed between the taking of the two samples. The first sample was drawn immediately after the induction of anesthesia.

The difference observed between the average figures in the two columns is not of sufficient magnitude to be significant since the range of experimental error for the method is 3 to 5 per cent. For reasons which have been discussed by Winslow, Falk and Caulfield (1923), some uncertainty attaches to the conversion of electrophoretic measurements in u/sec to millivolts. The *apparent* conversion factor for the data reported here is 1.3.

In table 2 are shown the results of determination on samples of blood drawn immediately after the induction of anesthesia and after an average of one and one-half hours' irradiation with a beam of 4000 f.c. intensity projected from a carbon arc and with a total spectrum, as shown in a previous paper (Reed, 1925a, fig. 4).

With one exception there was a consistent decrease in the rate in the

[1] National Research Council Fellow.

second sample. The difference between the averages of the two columns is great enough to be of significance.

It is apparent from the results of these experiments that light applied directly to circulating blood causes a small but definite decrease in the negative electrophoretic potential of red blood cells.

It is not possible at present to interpret the significance of this change.

TABLE 1

Figures in terms of μ per second rate of movement of cells in electrophoretic chamber

EXPERIMENT	FIRST SAMPLE	SECOND SAMPLE
1	14.8	14.2
2	10.1	10.5
3	11.1	11.1
4	10.8	11.1
5	13.6	13.3
6	8.9	8.3
7	8.1	8.0
Average.....	11.1	10.9

TABLE 2

Figures express μ per second movement of cells in the electrophoretic chamber

EXPERIMENT	FIRST SAMPLE	SECOND SAMPLE
1	12.4	12.1
2	9.7	9.3
3	11.0	10.3
4	12.6	12.6
5	10.3	8.7
6	13.6	11.8
7	17.0	16.1
Average.....	12.4	11.4

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CHOLESTEROL AND PHOSPHATIDE METABOLISM IN PREGNANCY

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A marked increase in blood cholesterol and phosphatides during the latter part of pregnancy has been well established in the case of man, and a similar increase has been generally assumed to occur at this time in the blood of other animals. We were surprised, therefore, to note that the blood of two rabbits, which we used in another investigation, showed during pregnancy, instead of the expected increase, a progressive *decrease* in blood cholesterol and phosphatides. These findings led us to undertake the work reported in this paper in the hope that some information might be obtained concerning the nature of the unusual lipoid metabolism of pregnancy. A detailed study of the cholesterol and phosphatide content of rabbit blood was made in 7 normal rabbits during pregnancy and lactation.

1. Blood cholesterol and phosphatide variations during pregnancy in normal rabbits.[1] The phosphatides of blood, (and later of bile) are determined by our modification of the Bloor and Bell and Doisy methods (1). Cholesterol content of blood is determined by the method of Myers and Wardell (2) and the same principles are used in the estimation of cholesterol in bile.

Four cubic centimeters of blood were obtained twice weekly from the marginal ear veins of the animals. This was sufficient for duplicate estimations of both cholesterol and phosphatides. The data of 6 of the 7 normal animals used are summarized in table 1. The observations on the seventh rabbit were made at a different time and at different intervals. To present it here another table would be required. The results obtained with this rabbit are similar to those obtained with the other six and it is therefore not given. Two representative experiments of the group of normal pregnancies are shown graphically in figure 1.

From table 1 it can be seen that a progressive decrease in the cholesterol and phosphatide content of blood takes place during pregnancy in normal rabbits, beginning 7 to 10 days after conception and reaching its minimum

[1] A preliminary report of some of the experiments reported in sections 1 and 2 was made before the American Society of Biological Chemists on December 28, 1923 (Journ. Biol. Chem., 1924, lix, xxv).

a few days before or at parturition. (The period of gestation in rabbits is usually 30 days.) The maximum decrease varied from 39 to 66 per cent of the non-pregnant level, for cholesterol, and in the case of the phosphatides from 42 to 62 per cent, the averages in both cases being about 55 per cent.

TABLE 1

Blood lipoids during and after pregnancy in normal rabbits

DATE	338		397		446		455		456		458	
	Cholesterol	Lipoid P	Cholesterol	Lipoid P	Cholesterol	Lipoid P	Cholesterol	Lipoid P	Cholesterol	Lipoid P	Cholesterol	Lipoid P
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
3- 9-23	85	9.4	90*	9.6	82	10.9	106*	9.1	118	13.0	83	10.4
3-13-23	87	10.4	95	9.2	82*	9.1	94	10.7	111	10.2	96	9.8
3-16-23	82	8.1	89	10.2	83	10.2	93	11.0	115*	9.3	92	10.0
3-20-23	78	10.1	90	10.4	72	10.3	81	10.2	82	9.1	91	9.8
3-23-23	56	7 4	88	8 5	79	8.8	81	9.4	100	9.2	94	8.9
3-27-23	59	7.4	73	9.4	77	9.7	76	9.1	97	9.0	75	8.6
3-30-23	33	5.0	70	8.1	?	7.9	68	8.1*	89	9.3	55	6.7
4- 3-23	46	4.0	57	6.7	62	6.4	68	6.5	74	6.3	68	6.8
4- 6-23	55	6.1	55	6.3	59	8.0	65	7.3	80	8.7	69	6.8†
4-10-23	70	8.0	59	5.9	59	6.3	65*	8.6	73	8.5	94*	9.3
4-13-23	82*	9.4	58	6.3	54	6.4	59	6.1	53	6.6	73*	8.0
4-17-23	75	8.3	63	9.2	72	8.1	54	5.2	52	5.7*	64	7.5
4-20-23	59	7.1	67	8.9	61	7.7	55	5.9	72	6.1	74	8.2
4-24-23	61	7.2*	53	7.7*	69	7.6*	84	10.6	83	8.4	64	8.6*
4-27-23	61	7.0	54	7.4	67	6.4*	70	9.0*		8.6	76	8.0
5 -1-23	67	7.3	65	8.5	67	7.4	65	10.0*	71	7.9	75	7.7
5- 4-23	72	9.5	54	11.1	65	8.6	75	10.5	82	9.2	82	9.4
5- 8-23	71	8.9	65	8.9	73	8.9	70	9.9	107	10.5	95	9.6
5-15-23	83	8.1	76*	9.5*	82	9.4*	72*	7.1	101	8.0	103	8.8
5-22-23	86	7.8	75	9.3	83	9.5	76	10.4	83	8.4		
5-25-23							70	11.7	126	8.9		
6- 1-23							82	8.3				

* Only one determination.

† Aborted on 25th day of pregnancy.

———Parturition.

Immediately after parturition there is a rather abrupt rise in the blood lipoids followed by a small drop which lasts for a week or ten days, giving place to a gradual rise to the non-pregnant level. This somewhat retarded return to the initial values might be ascribed to the loss of cholesterol and

FIGURE I

INFLUENCE OF PREGNANCY ON BLOOD CHOLESTEROL AND PHOSPHATIDES IN NORMAL RABBITS

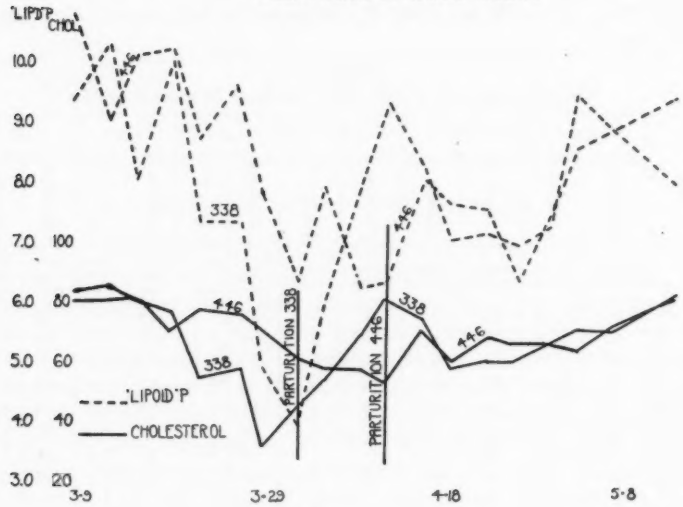
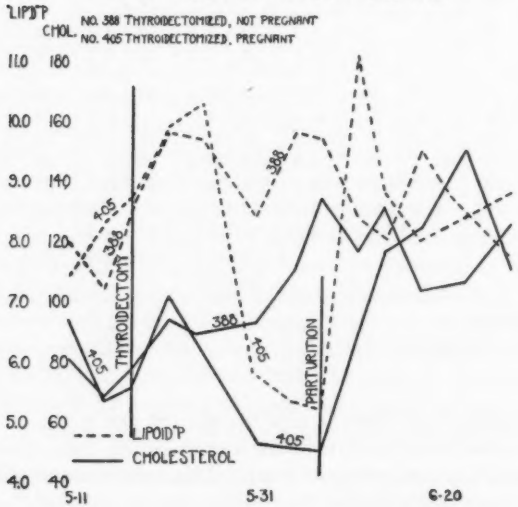


FIGURE II

INFLUENCE OF PREGNANCY ON BLOOD CHOLESTEROL AND PHOSPHATIDES IN THYROIDECTOMIZED RABBITS



phosphatides to the mammary secretion, but the same rate of return to the non-pregnant figures is noted in rabbit 458 which aborted on the 25th day of pregnancy and did not lactate (an observation which does not exclude, however, the possibility that lactation influences the level of blood cholesterol and phosphatides).

2. Blood cholesterol and phosphatide variations during pregnancy in thyroidectomized rabbits. In man thyroid enlargement often develops during pregnancy and a temporary relative thyroid deficiency may result. It has also been shown that in clinical thyroid insufficiency a lipoidemia is present. It might therefore be possible that the increased blood lipoids of human pregnancy are associated with thyroid deficiency.

We have been able to obtain evidence that a lipoidemia occurs in goitrous rabbits as well as in man, and in addition, have demonstrated that this lipoidemia is due to a thyroid deficiency. For when a rabbit that had an hyperplastic thyroid and also a lipoidemia, was fed a small amount of KI to involute the gland, the lipoidemia gradually diminished.[2] With these facts in mind, we studied the behavior of the blood lipoids in some thyroidectomized pregnant rabbits.

The data of four thyroidectomized rabbits that were bred 3 to 9 days before thyroidectomy are presented in table 2. One of these animals, 388, did not conceive and serves as an excellent control. The observations on this rabbit and on one of the pregnant thyroidectomized rabbits are shown graphically in figure 2. Similar observations were made during and after 3 pregnancies on two other animals whose thyroids had been almost completely removed. Results obtained were entirely like those of the first group and the data are therefore not given. One previously suprarenalectomized rabbit, no. 263, became pregnant while its blood lipoids were being studied and these data are also included in table 2.

As in cases of myxedema in man, a marked rise in blood cholesterol takes place in experimental myxedema also, reaching twice its normal value in two months or less. A more transient rise of blood phosphatides also results, which is smaller in degree. Comparison of these findings on thyroidectomized pregnant rabbits with the data on normal pregnant animals indicates that the influence of thyroidectomy delays the decrease in blood lipoids noted in normal pregnancy until 10 to 14 days before parturition. Then a rapid and marked decrease in both blood cholesterol and phosphatides is observed which is perhaps slightly greater in degree than that found in normal pregnant rabbits. Rabbit 444 aborted on the 27th day of pregnancy and this probably accounts for the fact that the decrease was not so great as in numbers 405 and 442.

None of these thyroidectomized animals nursed their young for more than a day or two so the influence of lactation in thyroidectomized rabbits

[2] Unpublished data.

cannot be reported on. (It is doubtful if *completely* thyroidectomized rabbits ever rear their young.) Within less than a week the blood lipoids in these rabbits rose to a level higher than the non-pregnant values and continued high.

In striking contrast to these three thyroidectomized rabbits, number 388 is presented; here the typical effects of thyroidectomy[3] uncomplicated

TABLE 2

Blood lipoids during and after pregnancy in thyroidectomized rabbits and a suprarenalectomized rabbit

THYROIDECTOMIZED									SUPRARENALECTOMIZED		
Date	388		405		442		444		Date	263	
	Cholesterol	Lipoid P	Cholesterol	Lipoid P	Cholesterol	Lipoid P	Cholesterol	Lipoid P		Cholesterol	Lipoid P
	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent		mgm. per cent	mgm. per cent
5-11-23	83	8.1	96	7.4	—	9.4	—	8.2	12-15-21	76	7.6
5-15-23	70	7.3	69	8.4	115	9.9	91	8.6	12-19-21	75	8.2
5-18-23	79	8.9	73	8.8	110	11.8	87	10.3	12-23-21	78	7.9
5-22-23	96	9.8	103	10.0	105	11.2	106	9.5	12 30-21	74	7.3
5-25-23	98	9.7	89	10.4	136	11.7	116	11.1	1- 5-21	79	
6- 1-23	95	8.5	54	5.9	94	8.6	104	9.2	1-11-22	62	7.3
6- 5-23	112	9.9	53	5.4	72	6.8	76	9.5	1-14-22	61	7.9
6- 8-23	136	9.8	52	5.4	66	6.7	73	7.4†	1-17-22	56	5.9
6-12-23	118	8.5	91	11.2	49	5.0	69	8.9	1-20-22	70	11.5
6-15-23	133	8.1	118	8.9*	64	6.8	126	6.8	1-24-22	73	
6-19-23	105	9.6	126	8.1	120	11.7	155	11.1	1-28-22	62	8.6
6-22-23	114	8.9	152	8.3	90	9.6	146	10.3	2-1-22	57	6.0
6-29-23	127	7.8	112	9.0	125	11.6	110	11.1			
7- 6-23	113			8.9	156	10.3	97	10.0			
7-18-23			104	10.0	206	11.0	154	10.8			
7-27-23			106		163		135				

* Only one determination.

† Aborted on 27th day of pregnancy.

————Thyroidectomy.

.....Parturition.

by pregnancy are seen. A steady rise to a maximum occurs 4 weeks after thyroidectomy. The cholesterol remains high while the comparatively slight rise of the blood phosphatides (20 per cent) lasts usually for six weeks or more.

[3] We have data showing similar rises in blood lipoids in other thyroidectomized rabbits.

The influence of pregnancy in decreasing the blood lipoids thus entirely overcomes the lipoidemia following thyroidectomy. Therefore, it seems unlikely that the lipoidemia of human pregnancy is dependent upon a lack of thyroid secretion.

In the one suprarenalectomized animal the data presented are not so detailed; they were not obtained with the idea of determining the influence of pregnancy on blood lipoids. Variations quite similar to those occurring in normal rabbits were observed in this animal so that suprarenalectomy is probably without influence on this phenomenon.

TABLE 3

Blood lipoids during and after pregnancy in dogs

DOG 1			DOG 2			DOG 3		
Date	Cholesterol	Lipoid P	Date	Cholesterol	Lipoid P	Date	Cholesterol	Lipoid P
1924	*mgm. per cent*	*mgm. per cent*	*1924*	*mgm. per cent*	*mgm. per cent*	*1924*	*mgm. per cent*	*mgm. per cent*
5-29	204	17.6	9-29	204	16.5	9-22	154	13.3
						1925		
6-3	Parturition		10-6	203	16.6	4-4	137	11.9
6-6	185	17.8	10-27	174	19.8*	4-15	135	12.0
6-17	89	9.0	10-30	178	19.2*	4-22	136	13.4
6-24	123	10.2	11-3	180	17.4	4-30	213	13.9
8-12	195	16.0	11-6	178	15.1	5-4	Parturition	
			11-10	Parturition		5-6	195	12.0
			11-10	200	13.2	5-13	142	10.2
			11-13	207	12.9	5-26	117	8.8
			11-17	187	12.0	6-5	113	7.4
			11-26	141	12.1	6-26	165	11.3
			12-1	149	12.3			
			12-8	166	14.1			
			12-23	183	14.0			
			1925					
			1-5	182	14.3			

* Slight vaginal infection.

3. Blood cholesterol and phosphatide variations during pregnancy in dogs. To aid in the elucidation of the questions raised by the results found in sections 1 and 2, it was desirable to discover how the blood cholesterol and phosphatides of a carnivore behaved during pregnancy. For this purpose the dog was chosen and up to the present we can present observations on only three. The results will be found in table 3.

The blood in all cases was obtained in the morning before the animals were fed. With dog 1 we were able to obtain only one specimen before parturition. In this case and in the case of dog 2 it is clear that there was no increase in the blood lipoids during pregnancy, nor was there a decrease

of any significance. Dog 3 however did show a definite rise in blood cholesterol and phosphatides for a period of about ten days just before and after parturition. This was a young animal pregnant for the first time. There were twelve in the litter of whom 6 were either born dead or died immediately after birth. The mother had been obviously uncomfortable for several days before delivery. Because of these facts we are inclined to look upon the increased blood cholesterol and phosphatides found just before the end of pregnancy in this case as abnormal. The usual findings in pregnant dogs are probably represented by the results obtained with dogs 1 and 2.

In each of these three animals a progressive decrease in the blood cholesterol and phosphatides occurred after parturition. These decreases are probably the result of lactation. Similar observations have been made on blood phosphatides in the lactating cow (3).

4. A comparison of the cholesterol and phosphatide content of maternal and fetal blood at various stages of pregnancy. Chauffard, Laroche and Grigaut (4) first observed the very low cholesterol content of blood from the umbilical cord (human). Klinkert (5), comparing the cholesterol content of maternal and fetal blood, noted the lower cholesterol concentration of the fetal blood. These observations were confirmed by Vercesi (6) by Huffman (7) and by Hymanson and Kahn (8) who suggested that very little cholesterol passes through the placenta, a concept further developed by Slemons, Curtis and Stender (9) who showed that the total fatty acids and phosphatides as well as cholesterol are present in greater concentration in maternal than in fetal bloods. These data have been confirmed by others.

We have made investigations on rabbits similar to those just quoted on man. Here again we obtained results quite the reverse of those found for man. We have in addition, partly by good fortune, been able to observe the influence of thyroid insufficiency on the maternal and fetal blood lipoids, for two of the animals used in this study had been partially thyroidectomized and two others proved to have spontaneous goiters—a condition that is rare on the sea coast.

Rabbits were sacrificed at various stages of pregnancy by a blow on the back of the head. The abdomen was opened at once and, after thoroughly drying off the amniotic fluid, blood was obtained from the embryos by cutting their jugulars and carotids. Attempts to obtain blood from the umbilical cord were unsatisfactory. Maternal blood had been obtained from a marginal ear vein shortly before sacrificing the animal. The maternal and fetal specimens were analyzed at the same time and *compared with the same standards.* The results are given in table 4.

Of these seven rabbits, one, no. 450, was sacrificed one day postpartum because of pneumonia. In this case the cholesterol and phosphatides were about 20 per cent *higher* in the blood of the mother than in the combined

specimens from the young. The other six rabbits were all sacrificed before parturition, from the 23rd to 29th day of gestation. Contrary to what has been found in man, all of the *fetal specimens showed a higher concentration of lipoids* than did the maternal bloods.

Of these six the three rabbits with normal thyroids (nos. 452, 456 and S 100) had about 25 per cent more cholesterol in the fetal blood than in the maternal blood while the phosphatides ranged from 12 to 28 per cent higher.

TABLE 4

Comparison of lipoid content of maternal and fetal blood

RABBIT NUMBER	STAGE OF PREGNANCY	CHOLESTEROL			LIPOID P			REMARKS
		Maternal blood	Fetal blood	Increase of fetal blood	Maternal blood	Fetal blood	Increase of fetal blood	
		mgm. per cent	*mgm. per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	
399	23rd day	64	142	122	7.0	9.6	37	Partial thyroidectomy
493	24th day	50	121	142	6.3	9.2	46	Goiter
494	24th day	58	123	112	7.3	12.0	64	Thyroid markedly enlarged. Smaller than 493
452	28th day	66	83	26	6.8	7.9	16	Normal
456	28th day	73	88	21	6.9	7.6	10	Normal
S100	29th day	68	87	28	6.1	8.0	31	Normal
450	1st day postpartum	156	122	−22	8.6	7.4	−14	Partial thyroidectomy

Necropsy notes: Space will not permit us to give full necropsy protocols.

The pertinent findings concern mainly the thyroid gland. Rabbits 452, 456 and S 100 had normal thyroid glands (no histological examination on S 100). Rabbits 399 and 450 had had partial thyroidectomies performed and the remaining fragments were moderately hyperplastic. No. 450 was sacrificed one day postpartum because of a clinical diagnosis of pulmonary disease. At autopsy, massive pneumonitis noted. Rabbit 493 and 494 had very markedly hyperplastic thyroids, greatly enlarged. In the case of 493 a real simple goiter was present, the thyroid being over twice as large as normal.

The three rabbits with hyperplastic thyroid glands that were sacrificed before term (nos. 399, 493, 494) showed similar changes but to a much more marked degree, the fetal blood-cholesterol exceeding the maternal value by well over 100 per cent and the fetal blood phosphatides 37, 46 and 64 per cent above the corresponding maternal blood figures.

In the rabbit the difference in composition between maternal and fetal blood is normally not nearly so great as in man and the difference is in the opposite direction. That is, instead of a lower cholesterol and phos-

phatide content in the blood of the fetus we find 25 per cent more than in the blood of the mother. The much greater differences observed in rabbits having a thyroid insufficiency will be discussed presently. For the moment it need only be pointed out that the differences are of the same sort as those found in "normal rabbits" but very much greater.

5. The cholesterol and phosphatide content of bile during pregnancy. To explain the hypercholesterolemia of human pregnancy, the theory of Bacmeister and Havers (10) has been frequently quoted. In studying cholesterol excretion during one pregnancy in a bile fistula dog, they found the cholesterol content of the bile decreased during the latter half of pregnancy. After parturition it rose again. From these observations they postulated their theory that during pregnancy the liver acted as a dam preventing the excretion of cholesterol through its usual channel and in this way caused an hypercholesterolemia. However, they did not report any data showing that there actually was such an hypercholesterolemia in the pregnant dog, nor could we find mention of any in the literature. From the few experiments that have been presented in section 3, it seems probable that no hypercholesterolemia is found during pregnancy in the dog.

Several workers have attempted to obtain information along these lines by determining the cholesterol content of bladder bile of pregnant women who have come to autopsy. McNee (11) reports an increase in the cholesterol content of the bile in pregnancy while others have found a decrease. These conclusions are open to objection in that death was often due to complications arising in pregnancy.

More recently Pribram (12) from analyses of "bile" obtained with a duodenal sound from women during pregnancy and after parturition believes he has shown that there is less cholesterol in the bile of pregnant than in that of parturient women. Waiving the question of unequal dilution of bile in the duodenum by gastric and intestinal secretions, we do not believe Pribram's data support his conclusions. If one takes the average of *all* the observations on the 15 pregnant and compares that with the average value obtained from the bile of the 12 parturient women there is a definite increase in bile cholesterol in the latter group. But, the individual figures vary so widely that one is not justified in averaging such data. If one compares the cholesterol content of bile on the six cases in which Pribam was able to get specimens both before and after parturition, one finds no change in 3 cases, a great *decrease* after parturition in one case and smaller increases in two cases. From such results a conclusion that less cholesterol is excreted in the bile of pregnant women is not warranted. Figures such as 84 and 120 mgm. of cholesterol per 100 cc. of blood which he reports for two women in the 9th month of pregnancy are unusually low for normals.

We have analyzed the bile of pregnant and non-pregnant rabbits. The data are presented in table 5. The composition of bladder bile is highly variable. The first 3 animals in the table were pregnant and all had marked thyroid hyperplasias. The thyroids of the other six were not hyperplastic. Three of these were not pregnant. The remaining three were pregnant when sacrificed. As might be expected because of the concentration that bladder bile undergoes, there are rather wide variations in its composition, but even when calculated on dry basis the variations, especially of the phosphatides, are great. There is no definite trend in any direction.

6. The plasma CO_2 content of pregnant rabbits. At this stage of this investigation we had become convinced that none of the various theories

TABLE 5

The cholesterol and phosphatide content of bile of normal and pregnant rabbits

NUMBER	PER CENT H_2O	PER CENT CHOLESTEROL	PER CENT PHOSPHATIDE	PER CENT OF BILE SOLIDS		REMARKS
				Cholesterol	Phosphatide	
399	79.5	0.10?	5.1	0.49	24.8	Pregnant 23 days—partial thyroidectomy
493	81.5	0.10?	3.3	0.54	17.9	Pregnant 24 days—marked thyroid hyperplasia
494	76.8	0.10?	4.9	0.43	21.2	Pregnant 24 days—marked thyroid hyperplasia
452	75.6	0.22	8.0	0.90	32.8	Pregnant 28 days
456	78.6	0.18	10.3	0.84	48.1	Pregnant 29 days
100	75.6	0.14	1.6	0.57	6.6	Normal
445	79.5	0.14	5.8	0.68	28.3	Normal
481	85.9	0.16	0.60	1.14	4.26	Normal—young 7 months normal
112	88.0	0.12	0.50	1.0	4.2	

that had been advanced to account for the hypercholesterolemia of human pregnancy were adequate and we were coming to believe that the lipoidemia might be the response to a mild intoxication that normally occurs in the latter part of human but not in rabbit pregnancies. The diminished alkaline reserve that Hasselbach (13) found in late pregnancy in man, which has been repeatedly substantiated since by Losee and Van Slyke (14) and many others, could easily be conceived to be another manifestation of this intoxication. If pregnant rabbits do not have this postulated toxemia, then they would very likely not show this early indication of a disturbance of the blood reaction found in man.

We put this question to the test by determining the CO_2 content of plasma of 4 non-pregnant rabbits and 3 pregnant rabbits on their 28th, 29th and 30th days of pregnancy respectively. Blood was drawn from the

marginal ear veins of rabbits in the usual way for estimation of the CO_2 content of plasma by the Van Slyke method. It was found very difficult to obtain satisfactory specimens unless the rabbit had rested about 20 minutes and did not struggle while the blood was drawn. If struggling did occur the CO_2 values were of course low. Such specimens can not be used. The results are shown in table 6.

The plasma CO_2 values of the normal rabbits varied between 53.0 and 56.8 cc. CO_2 per 100 cc. plasma while the range for the pregnant animals lay between 53.0 and 58.7 cc. CO_2 per 100 cc. plasma. The data are similar to those found by Blatherwick (15) on the pregnant and non-pregnant cow. He likewise found no change in the alkaline reserve during pregnancy in the cow. These figures show that the low CO_2 content of plasma obtaining in pregnant women is not found in pregnant rabbits and so give some support to our hypothesis.

TABLE 6

The plasma CO_2 content of non-pregnant and pregnant rabbits

NUMBER	AGE	INTERVAL SINCE BRED	CO_2 PER 100 CC. PLASMA
	months	*days*	
470	22	0	56.8
464	19	0	53.0
474	22	0	54.1
482	22	0	56.8
471	18	28	54.6
549	10	29	58.7
550	9	30	53.0

DISCUSSION. Our experiments show unequivocally that a gradual but marked decrease in blood cholesterol and phosphatides amounting to about 55 per cent occurs during pregnancy in the rabbit. This is quite the reverse of what has been repeatedly observed in the case of pregnant women and of what has been usually assumed to occur in other mammalia. In the dog there is probably no rise in blood lipoids during pregnancy; probably a slight decrease. So far as we have been able to find out, no studies on the blood phosphatides or cholesterol during pregnancy, have been made on any animal except man and the cow. Meigs, Blatherwick and Cary (3), in a paper on phosphorus and calcium metabolism and its relation to milk secretion noted a definite decrease in lipoid phosphorus of blood during the latter part of pregnancy in cows. They do not offer any definite explanation of this phenomenon although they believe as a result of other experiments that the phosphatides play a very direct and important rôle in the secretion of fat in milk.

The lipoidemia of human pregnancy has been explained by some as a

preparation for lactation notably Herrmann and Neumann (16). If this explanation is valid one should expect to find a similar blood picture in the pregnant cow—an animal in which the function of lactation has been most highly developed—but apparently one does not. Our results on rabbits and dogs indicate that the findings in man are exceptional.

The theory proposed by Havers and Bacmeister, namely, that lipoidemia of pregnancy is caused by the liver acting as a dam and so preventing the usual excretion of cholesterol through the bile, is based on a study of bile cholesterol excretion in a dog but no blood cholesterol experiments were made on this animal. Probably there was no hypercholesterolemia. In the pregnant rabbit, we have found no evidence of decreased cholesterol excretion in bile, while in man there is likewise no evidence indicating that less cholesterol is excreted during pregnancy. Besides, much of the cholesterol excreted in the bile is probably reabsorbed, so variations in the amount of cholesterol of bile should hardly cause great differences in cholesterol metabolism in the body.

Comparison of human maternal and fetal blood shows that the amount of lipoids is higher in the maternal than in fetal blood. From such observation and also from the fact that fat soluble dyes do not pass through the mammalian placenta, several investigators have supposed that the lipoidemia of human pregnancy may be due, at least in part, to an impermeability of the placenta to these lipoids, which may result in their accumulation in the maternal blood. To us, such a theory does not seem likely. There is no decrease in the maternal protoplasmic mass. Granting that the placenta is impermeable to lipoids, the addition of 5 per cent of protoplasm can hardly be the cause of an increase in blood phosphatides and cholesterol of 25 per cent or more on such a basis alone.

The placenta of rabbits is essentially similar to that of man. We have found that Sudan III and Scarlet Red[4] will not pass through the rabbit placenta. Yet in the rabbit, the lipoids are higher in the fetal than in the maternal blood. The divergence in both man and the rabbit is due to *variations from the normal, in the maternal blood, during pregnancy.* The lipoid content of human and rabbit fetal blood is similar to that found in the young. Even when a condition such as thyroid deficiency exists in a pregnant rabbit, we have seen that the blood lipoids of the embryos increase in exactly the same way as they would in any goitrous animal although the maternal blood lipoids *during the pregnancy* do not. This atypical behavior of the *mother* ceases at parturition.

The different dietaries of omniverous and herbivorous animals may account for some of the differences observed in the behavior of the blood lipoids in pregnancy in man and in the rabbit, dog and cow. No doubt, to the low cholesterol content of the rabbit's diet can be ascribed the low

[4] Unpublished experiments.

blood cholesterol values found in this species when compared with that of man or the dog. The subnormal blood lipoid figures found in the latter part of pregnancy in rabbits could readily be due to a more extensive utilization of these substances during the period of very active growth and differentiation of the embryos, mammary gland, uterus etc.

It does not, however, seem likely that the food factor alone could adequately account for the differences in behavior of blood lipoids observed in man and in the rabbit. The dietary in each is essentially the same before, during and after pregnancy. In each case we have seen that the fetal blood composition varies in the way characteristic of each species under several conditions but composition of the maternal bloods undergoes changes which vary strikingly from each other and also from their normals.

It may be that the lipoidemia of pregnancy in man is due to an intoxication that does not occur in rabbit pregnancy. It is well known that in any kind of intoxication—bacterial or chemical—a lipoidemia occurs. The maternal circulation carries off various waste products from the fetus, some of which are probably toxic. In the early stages of pregnancy in man these toxic waste products may be satisfactorily disposed of but as the fetus grows and they increase in amount the mother has difficulty in completely excreting or detoxicating them, and like any other form of intoxication, this should cause a lipoidemia. It is well known that in the toxemias of pregnancy in man one always finds that the cholesterol and phosphatide content of blood are very much higher than the values found in normal human pregnancies. Some support of this view is given by the lowered CO_2 content of plasma that is found during pregnancy in man but which does not occur in pregnant rabbits. Our experiments on dogs are too limited to draw definite conclusions but they indicate that an intoxication very likely occurred in the only dog that showed a rise in blood lipoids for a few days just before parturition.

What rôle the mobilization of these substances plays we do not know at present. However, cholesterol and phosphatides have the power of combining with several toxins such as cobra venom, saponins etc., and so detoxicate them.

If we accept this hypothesis for the moment, it would still be necessary to explain why in human pregnancy one finds an increased content of lipoids in the blood while in rabbits the reverse is observed. Toxemias of pregnancy are far more common in man than in other animals. In over 100 litters of rabbits from normal mothers that have been raised in this laboratory we have never seen any toxemia. When any irregularity in development occurs during pregnancy abortion takes place more promptly than in man. No doubt with a wide enough experience one will find toxemias of pregnancies in rabbits and in such cases we should expect to find an increased content of lipoids in the blood.

As a working hypothesis, we suggest that the lipoidemia of pregnancy in man is a secondary reaction called forth by a mild intoxication. These phenomena are not normally found in rabbits and probably not in cows or dogs either.

SUMMARY

Unlike what occurs in man, in the case of the rabbit the blood cholesterol and phosphatides decrease during the last half of pregnancy to nearly half the non-pregnant levels. Thyroid deficiency in rabbits causes the same rise in blood cholesterol and phosphatides observed in man. In spite of this rise, when a thyroidectomized or goitrous rabbit becomes pregnant, the influence of pregnancy completely overcomes this lipoidemia, and a decrease in blood cholesterol and phosphatides quite similar to that occurring in normal rabbits is found.

In dogs there seems to be little change in blood lipoids in normal pregnancies.

Contrary to what is found in man, the blood of rabbit embryos has a higher cholesterol and phosphatide content than that of their mothers. It is pointed out that the values found in fetal blood are of the same magnitude as those found in normal non-pregnant rabbits and that the fetal blood cholesterol in man approximates the values found in children. Further, the embryos of rabbits suffering from thyroid insufficiency react in the way that an adult would—that is, they have an increased amount of cholesterol and phosphatides in the blood—in spite of the maternal blood lipoid values being subnormal.

Analysis of the bladder bile of normal and pregnant rabbits lends no support to the theory of Bacmeister and Havers that the liver acts as a dam toward cholesterol during pregnancy, preventing its excretion through this channel. In addition, reasons are given for holding this theory as well as other theories that have been advanced to explain the hypercholesterolemia of human pregnancy, such as impermeability of the placenta, preparation for lactation etc., etc., inadequate.

We are inclined to explain the lipoidemia of human pregnancy as a reaction similar to those called forth by any intoxication and reasons for the different findings in man and the rabbit are suggested.

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CHOLESTEROL AND PHOSPHATIDE DISTRIBUTION IN SOME TISSUES OF PREGNANT AND NON-PREGNANT RABBITS

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In the previous paper (1) it was shown that during pregnancy in rabbits there is a large reduction in blood cholesterol and phosphatides. Attempts were made to determine the nature of this phenomenon and possible explanation for the striking difference in the behavior of the blood lipoids in pregnancy of the rabbit and man was suggested. In this report the fate of the cholesterol and phosphatides that are removed from the blood during pregnancy in the rabbit is investigated.

Methods: The tissues were finely minced in a special machine and a weighed portion was mixed with plaster of Paris and dried in a vacuum desiccator over sulphuric acid for 3 or 4 days. This dried cake was again ground in a mortar with powdered glass and extracted in a Wiley extractor for three days, using anhydrous ether and alcohol alternately as previously described (2). The extract dried was subsequently taken up in chloroform. Duplicate estimations of cholesterol and lipoid phosphorus were made on this chloroform solution.

To show the completeness of extraction, table 1 is submitted. The tissues used in these cases were rabbit and ox kidneys. Similar results have been obtained with muscle and other tissues. Since cholesterol is much more easily extracted than phosphatides the cholesterol determinations that were made on these extracts are not included in the table. The 3-day-extraction process almost always yielded 95 per cent or more of the total lipoids extractable in 10 or 12 days.

1. The cholesterol and phosphatide contents of embryos, placentas and amniotic fluid. To get an idea of the amount of cholesterol, phosphatides and fat in embryonic tissues the fetuses of 4 rabbits were analyzed, and the results are presented in table 2. The placentas and amniotic fluids of these rabbits were finely hashed, mixed, and the cholesterol and phosphatides determined as shown in table 3.

The total amount of cholesterol in the embryonic tissue of the largest of these 4 rabbits is thus found to be 0.86 gram while the corresponding quantity of phosphatides is 2.10 grams. The usual maximum decrease in blood cholesterol during pregnancy was about 40 mgm. per 100 cc. (see

preceding paper). The total blood in this rabbit calculated from its weight would be about 175 cc., so the total decrease in the cholesterol content of the blood would be 0.07 gram which at a given time represents only a small proportion of the cholesterol in the embryonic tissues. The

TABLE 1

Showing completeness of extraction process

NUMBER	WEIGHT OF CHARGE	WEIGHT OF FAT EXTRACTED IN		WEIGHT OF LIPOID P EXTRACTED IN		PER CENT OF TOTAL FAT EXTRACTED IN		PER CENT OF TOTAL LIPOID P EXTRACTED IN	
		1–3 days	4–12 days	1–3 days	4–12 days	1–3 days	4–12 days	1–3 days	4–12 days
	grams	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	*per cent*	*per cent*
1	1.755	69.6	0.8	1.067	0.001	98.8	1.1	99.9	0.1
2	1.582	52.4	3.2	0.833	0.032	94.3	5.7	96.3	3.7
3	1.905	77.0	2.4	1.486	0.023	96.8	3.2	98.5	1.5
4	1.755	47.8	3.6	0.825	0.044	93.2	6.8	94.9	5.1
5	2.599	98.2	2.6	1.690	0.028	97.4	2.6	98.4	1.6

TABLE 2

Lipoid distribution in rabbit embryos

NUMBER	WEIGHT	LENGTH OF PREGNANCY	WEIGHT OF EMBRYOS	WATER	"FAT"*	CHOLESTEROL	PHOSPHATIDES
	grams	*days*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*
452	2482	20	98	84.9	2.95	0.24	0.56
456	3080	28	87	85.4	3.21	0.17	0.56
516	3175	28	201	82.1	5.33	0.20	0.65
549	3490	29	280	82.3	4.36	0.23	0.64

* Total ether-alcohol extract soluble in chloroform minus cholesterol and phosphatides.

TABLE 3

Lipoid distribution in combined placentas and amniotic fluids of rabbits

NUMBER	WEIGHT OF PLACENTA AND FLUID	WATER	"FAT"*	CHOLESTEROL	PHOSPHATIDES
	grams	*per cent*	*per cent*	*per cent*	*per cent*
456	60	90.6	1.04	0.36	0.31
516	59	88.0	1.31	0.30	0.60
549	59	86.7	1.37	0.27	0.66

* Total ether-alcohol extract soluble in chloroform, minus cholesterol and phosphatides.

total decrease in blood phosphatides would be 0.18 gram which could similarly account for only a small part of the 2.1 gram of phosphatides of the embryos. If the cholesterol and phosphatides of the embryos were obtained from the mother, they would obviously be supplied continuously

so that the decrease in maternal blood lipoids could be due to a transfer of these substances to the embryos.

As noted in the previous paper, however, what little evidence there is, indicated that the placenta is impermeable to lipoids or at least that no very active diffusion of them takes place through it. Recent evi-

TABLE 4

Lipoid distribution in mammary glands of pregnant rabbits

NUMBER	LENGTH OF PREGNANCY	WATER	"FAT"*	CHOLESTEROL	PHOSPHATIDES
	days	*per cent*	*per cent*	*per cent*	*per cent*
452	20	70.5	6.8	0.18	0.40
414	22	64.7	13.1	0.21	0.43
399	23	70.1	12.3	0.17	0.88†
493	24	60.8	20.3	0.18	0.85†
494	24	57.3	23.5	0.20	1.70†
S100	28	61.6	20.7	0.15	0.72
450	1 pp	70.3	15.5	0.17	0.53
440	17 pp	58.7	20.7	0.22	0.65

* Total ether-alcohol extract soluble in chloroform minus cholesterol and phosphatides.

† These rabbits had hyperplastic thyroid glands.

pp, post-partum.

TABLE 5

Lipoid distribution in mammary glands of pregnant rabbits on dry basis

NUMBER	"FAT"*	CHOLESTEROL	PHOSPHATIDES
	per cent	*per cent*	*per cent*
452	22.6	0.60	1.33
414	37.1	0.60	1.22
399	41.1	0.57	2.94†
493	51.8	0.46	2.17†
494	55.0	0.47	3.98†
S100	53.9	0.39	1.88
450	52.3	0.57	1.79
440	50.1	0.53	1.58

* Total ether-alcohol extract soluble in chloroform minus cholesterol and phosphatides.

† These rabbits had hyperplastic thyroid glands.

dence shows, moreover, that the embryo is capable of synthesizing its own supply of cholesterol and phosphatides.

2. The cholesterol and phosphatide contents of mammary gland. The embryos and placentas comprise the new tissues which are formed during pregnancy. Several other organs enlarge markedly at the same time, notably the uterus and mammary glands. To gain an idea of the changes

that occur in these tissues, mammary gland was analyzed at various stages of pregnancy from the 20th day of gestation, when there is little noticeable change in the mammary gland on gross examination to the 29th day of gestation. Two post-partum specimens were also examined, one the first day and the other on the 17th day after parturition. The results are found in table 4 and calculated on a dry basis in table 5.

At autopsy three of these rabbits, nos. 399, 493 and 494, were found to have hyperplastic thyroid glands (gross and microscopic examination) the most marked instance being no. 494, in which the condition amounts to a definite simple goiter.

From these data there seems to be no definite change in the percentage of cholesterol in the mammary gland as pregnancy advances. The per cent of "fat" and of phosphatides calculated on either wet or dry basis increases markedly. At the end of pregnancy the per cent of fat is two or three times as high as it is on the 20th day, while the phosphatides are nearly twice as high. In the animals having deficiency of thyroid function, the increases in per cent of fat and the phosphatides are even more noteworthy.

If we estimate that the mammary glands of a rabbit increase in size from 10 to 75 grams during pregnancy, and we assume that the lipoids increase from the level represented by rabbit 452 on the 20th day of gestation to that of rabbit S 100 on the 28th day of gestation, then there would be 0.018 gram of cholesterol and 0.04 gram of phosphatides in the involuted mammary gland and 0.11 gram of cholesterol and 0.54 gram phosphatides at parturition. In the animals with thyroid insufficiency, differences in the amounts of cholesterol and phosphatides at the beginning and end of pregnancy are much greater.[1] In normal rabbits therefore, we find an increase of about 0.1 gram cholesterol and 0.5 gram phosphatides in the mammary glands during pregnancy, while the total decrease of these substances in the blood at the time of maximum decrease is 0.07 gram cholesterol and 0.18 gram phosphatides. This newly formed mammary tissue accounts for one and a half times as much cholesterol as the maximum decrease observed in the blood at a given time and for three times as much phosphatide. Considerable cholesterol and phosphatide would be required in the formation of the new tissue of the uterus and possibly to a smaller degree in other tissues (abdominal musculature, ovaries, etc.).

While the withdrawal of lipoids from the blood is taking place new cholesterol and phosphatides must be continuously formed. In the formation of new tissue a greater amount of lipoids seems to be required than can be supplied by synthesis and by the small amount contained in the rabbit dietary. As a result some of these substances present in the blood

[1] In this connection it is important to note that the decrease in blood cholesterol and phosphatides in rabbits having a thyroid deficiency, is somewhat greater than in normal rabbits.

are used at a more rapid rate than that at which they can be replaced. Such an explanation would adequately account for the hypocholesterolemia and hypophosphatidemia noted in pregnant rabbits.

TABLE 6

Analyses of hearts of normal rabbits

NUMBER	WEIGHT	AGE	"FAT"*	CHOLESTEROL	PHOSPHATIDES
	grams	*months*	*per cent*	*per cent*	*per cent*
410	1975	7	1.5	0.14	1.5
419	1937	7	1.5	0.15	1.7
421	2190	7	1.3	0.15	1.1
422	2940	7	1.4	0.12	1.1
481	2441	9	2.9	0.12	1.5
S A	2850	?	1.5	0.11	1.3
S B	2197	8	1.6	0.11	1.0
S107	1800	5	1.4	0.16	1.8
Average			1.64	0.133	1.38

* Total ether-alcohol extract soluble in chloroform minus cholesterol and phosphatides.

TABLE 7

Analyses of hearts of pregnant rabbits

NUMBER	WEIGHT	AGE	"FAT"*	CHOLESTEROL	PHOSPHATIDES	DURATION PREGNANCY	REMARKS
	grams	*months*	*per cent*	*per cent*	*per cent*	*days*	
399	3215	18	2.0	0.11	1.6	23	Thyroid hyperplasia
493	2780	11	1.9	0.15	2.0	24	Marked thyroid hyperplasia
494	3310	11	0.9	0.11	1.7	24	Thyroid hyperplasia
516	3175	20	2.5	0.12	1.6	28	
456	2903	13	2.3	0.20	1.5	28	Chronic snuffles
471	3100	18	2.5	0.10	1.8	28	
S100	3000	13	2.2	0.13	1.4	29	
549	3490	11	2.1	0.10	1.8	29	
543	3252	13	2.5	0.11	1.7	30	
450	2210	13	1.8	0.13	1.7	1 pp†	
Average			2.07	0.126	1.68		

* Total ether-alcohol extract soluble in chloroform minus cholesterol and phosphatides.

† Post-partum.

3. The cholesterol and phosphatide contents of pregnant and non-pregnant rabbit heart and kidney. The distribution of fats, cholesterol and phosphatides of non-pregnant and pregnant rabbit hearts and kidneys was also determined. Heart was chosen as an example of a tissue that might be

expected to have a pretty definite composition while kidney tissue would probably show changes more rapidly.

The ventricles were dissected and all visible fat removed, then hashed and analyzed. The results of these analyses on normal rabbits are found in table 6 and on pregnant rabbits in table 7.

TABLE 8

Analyses of kidneys of normal rabbits

NUMBER	"FAT"*	CHOLESTEROL	PHOSPHATIDES
	per cent	*per cent*	*per cent*
410	2.1	0.42	1.7
419	2.4	0.46	2.0
421	3.2	0.45	1.5
422	2.3	0.42	2.0
481	4.3	0.47	1.2
S A	1.9	0.40	1.7
S B	2.1	0.41	1.5
S107	1.9	0.51	1.9
Average.....	2.53	0.443	1.69

* Total ether-alcohol extract soluble in chloroform minus cholesterol and phosphatides.

TABLE 9

Analyses of kidneys of pregnant rabbits

NUMBER	"FAT"*	CHOLESTEROL	PHOSPHATIDES
	per cent	*per cent*	*per cent*
399	2.2	0.31	?
493	5.7	0.39	1.7
494	5.1	0.39	2.0
516	4.9	0.35	1.7
417	5.1	0.45	1.9
S100	5.3	0.45	1.3
549	3.7	0.33	1.8
543	4.4	0.35	1.8
450	3.7	0.37	1.4
Average.....	4.46	0.377	1.70

* Total ether-alcohol extract soluble in chloroform minus cholesterol and phosphatides.

The kidneys were prepared for analyses by stripping the capsules and removing the vessels in the hilus and hashing the remainder. The results are found in tables 8 and 9.

In the heart there is a definite increase in the per cent of "fat" during pregnancy. This becomes indeed striking if two observations (rabbits

481 and 494) which are out of line with the other figures, are not included in the averages. Then the average "fat" content of normal heart would be 1.46 per cent with a percentage deviation of 5.3 while that of pregnant heart would be 2.20 per cent with a percentage deviation of 10, the average increase being 1.38 per cent with a percentage deviation of 18. The corresponding figures for pregnant heart are 1.68 per cent and 7. These figures suggest a tendency toward increase in phosphatides in the rabbit heart during pregnancy.

No change in the cholesterol content of heart is found. Rabbit 456 which had chronic snuffles before and during the pregnancy showed a higher cholesterol value than any of the others. In a few instances we have noted similar values in hearts of animals that died of various infections.

As with heart tissue, kidneys of pregnant animals have a much higher "fat" content than is found in the non-pregnant. If no. 481 is not included in the average, as was done in the discussion of heart "fat" values, the average "fat" content of non-pregnant and pregnant rabbit kidneys would be respectively 2.27 per cent and 4.46 per cent—an increase of nearly 100 per cent. The per cent of phosphatides is unchanged while cholesterol figures for the kidneys of pregnant rabbits are slightly but fairly consistently lower.

SUMMARY

The "fat," cholesterol and phosphatide values of rabbit embryos, of placentas and amniotic fluid, of mammary glands at various stages of pregnancy and heart and kidneys of pregnant and non-pregnant rabbits have been determined.

The increase in the total amount of cholesterol and phosphatides in the mammary gland during pregnancy, together with similar but smaller increases that should occur in other tissues such as the uterus, ovary, etc., are shown to be sufficient to account for the decrease in blood cholesterol and phosphatides that occurs during pregnancy.

The thyroidectomies and anatomic examinations of the thyroid glands described in this and the preceding paper were made by Dr. David Marine. We are glad to express our indebtedness to him for this help.

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THE EFFECT OF PANCREATECTOMY ON THE SECRETION OF SUCCUS ENTERICUS

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In this study we have attempted to ascertain if a relation exists between the pancreas and the secretion of succus entericus.

That some relation might exist is suggested by several facts. In the first place we know that pancreatic juice and succus entericus contain enzymes of a similar general nature. Secondly, it is known that dogs following a successful ligation of the pancreatic ducts or pancreatectomy with insulin administration maintain their nutrition surprisingly well. This suggests that some compensatory change might occur in the succus entericus. Thirdly, the observations of a number of investigators have shown that the pancreas is in some way concerned in the regulation of blood and urinary diastases. (Schlesinger, 1908; Carlson and Luckhardt, 1908; Otten and Galloway, 1910; Wohlgemuth and Noguchi, 1912.)

METHODS. In our work we have used Thiry and Thiry-Vella fistula dogs. The quantity of the secretion was observed as well as the concentration of invertase, amylase and lipase. We collected the spontaneous secretion from the fistula and also the secretion excited by the subcutaneous injection of 4 mgm. of histamine (Koskowski, 1925).

The ferments were studied immediately after collection, the secretion being ground with glass to liberate intracellular enzymes. The following method was used for invertase determination: Four grams of saccharose (Merck) were dissolved in 100 cc. of phosphate buffer solution (Na_2HPO_4 with KH_2PO_4) with a pH of 6.0 which is the optimum H-ion concentration for the invertase of succus entericus of dogs. One-half of a cubic centimeter of succus entericus was added to 100 cc. of the above solution. A small amount of NaCl was added to the solution, and a few drops of toluol were added to prevent bacterial action. The mixture was placed in the incubator for 24 hours at a temperature of 40°C. For a control, a similar tube was prepared, immediately centrifugalized and the rotation determined. For amylase, we used Robert's method. For lipase we used the ethyl butyrate method.

[1] Rockefeller Foundation Fellow.

The diet consisted of 250 grams of bread and water. The first two days after pancreatectomy milk only was given.

Before pancreatectomy from 4 to 12 determinations were made on each dog, five dogs being used. After pancreactectomy determinations were

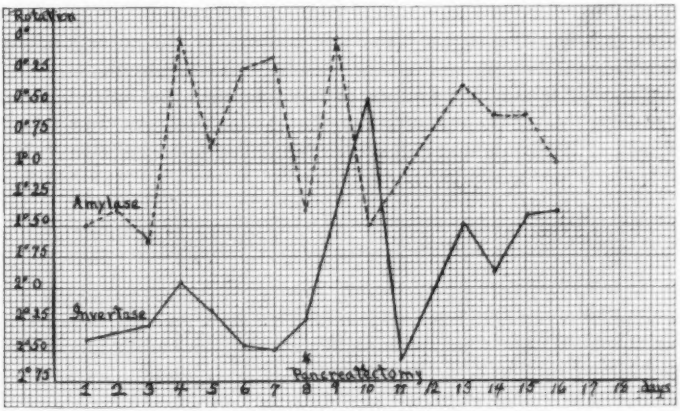

Fig. 1

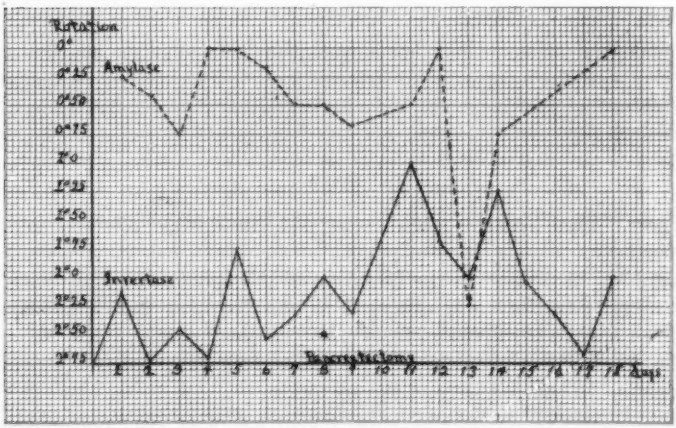

Fig. 2

made almost daily. The same interval of time was maintained between the collection of secretion in each dog before and after pancreatectomy.

Five dogs were studied.

RESULTS: *Concentration of ferments.* The results are shown diagrammatically in figures 1 to 3.

Invertase. Before pancreatectomy the invertase content of the secreti n was relatively constant. From 1 to 3 days after pancreatectomy there is a marked increase, after which in all but one dog there was almost complete disappearance of invertase, which in turn was followed by definite fluctuations or irregularities in the invertase content of the secretion, with a tendency for the average content to be slightly above normal.

Amylase. The amylase content of the secretion was quite variable in all the dogs. After pancreatectomy the amylase content was either not influenced (2 dogs); or was definitely diminished for from 2 to 7 days (3 dogs) followed by a return to normal.

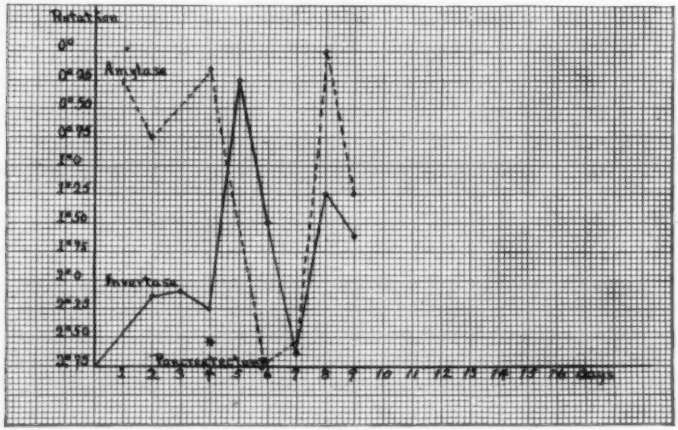

Fig. 3

Lipase. The lipase content was not materially affected by pancreatectomy.

Quantity. A change in the quantity of the spontaneous secretion and "histamine secretion" was observed in one dog (dog 2). This increase occurred only in the first two tests after pancreatectomy.

The increase in invertase concentration following pancreatectomy is not related to the quantity of the secretion.

Diet. Although we maintained a constant diet in our experiments, we varied the diet in dog 5 and even instituted several days of fasting without affecting the ferment content of the secretion.

DISCUSSION. Our results demonstrate that the only appreciable changes that occur in the secretion of succus entericus following removal of the pancreas are the changes in the concentration of invertase and amylase.

That the concentration of amylase in succus entericus is temporarily diminished following pancreatectomy is not surprising when viewed in the light of the fact that blood and urinary diastases are (King, 1914) temporarily diminished. This might be interpreted as meaning that the same unknown factors which control the concentration of blood and urinary diastases are concerned in the control of the amylase found in the intestinal juice.

The increase in the invertase in the succus entericus following pancreatectomy certainly shows that the invertase of succus entericus is not formed by the pancreas. This observation is difficult to explain because our knowledge of the origin of invertases is uncertain, as so many discrepancies appear in the literature. Normally invertase is absent from the blood or present only in traces Oppenheimer (1924), but Weinland (1906), and Abderhalden (1914) report that after an intravenous injection of cane sugar invertase appears, or is increased, in the blood. This latter point is disputed by Kuriyama (1916) and others (see Oppenheimer). We have been unable to follow the blood in our work, which however we will do later. Boisevain (1918) believes that invertase comes from leucocytes. Euler and his colleagues (1921) report that the invertase of leucocytes is different from the invertase of succus entericus in that it acts at a different pH. Since we used in our work the optimum pH for the invertase in succus entericus, we do not believe that the increase in invertase observed by us was due to a leucocytosis. This is supported by the fact that we failed to observe an increase in the invertase of succus entericus during the presence of an abcess in one of our dogs. The most reasonable explanations for the increase in the invertase in succus entericus which is found after pancreatectomy that occur to us are: *a*, that the biological disturbances that result following the removal of the pancreas affect the cells of the intestinal mucosa in some manner as to cause them to form more invertase, since there is no apparent increase in the number of cells in the succus entericus following pancreatectomy; or *b*, that the pancreas normally excretes invertase, preventing it from accumulating in the blood, so that when it is removed, the invertase of the blood is increased, which causes an increase in the invertase of the succus entericus. It is possible, however, that the pancreas in some manner inhibits the formation of invertase. Hence, when it is removed, the invertase content of succus entericus is increased.

Since the lipase content of succus entericus is unaltered by pancreatectomy, this shows that the formation of this ferment is in no way related to the pancreas.

Our observations fail to confirm the hypothesis that following pancreatectomy some compensatory change occurs in the secretion of succus entericus, which renders the animal more able to cope with the disturbance

of intestinl digestion due to the withdrawal of the pancreatic juice. It is still possible, however, that some compensatory change might occur after the first three weeks following pancreatectomy, as we did not follow our dogs longer than this period.

CONCLUSIONS

1. Diet or starvation for from 2 to 4 days does not affect the ferment concentration of the succus entericus.

2. Total pancreatectomy does not materially affect the quantity of the secretion of succus entericus.

3. The invertase content is definitely increased for 3 to 7 days, but fluctuates, and then there is a tendency for it to return to a level slightly above normal.

4. Total pancreatectomy causes a temporary decrease in the amylase content of succus entericus, but has no effect on the lipase content.

5. For the first 18 days following pancreatectomy no compensatory changes occur in the secretion of succus entericus which would facilitate intestinal digestion.

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STUDIES IN THE NUTRITION OF THE WHITE MOUSE[1]

I. The Normal Growth and Nutritive Requirements

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The white rat has been the experimental animal used in most of the recent nutrition investigations. Some of the reasons for the preëminence of this animal in this connection have been pointed out by Osborne and Mendel (1911a). The rat and the mouse are closely related species, but the latter has not been so extensively studied as has the former. The mouse has a smaller absolute food requirement, shows more rapid growth, is easier to handle, and its rapid rate and regularity of reproduction readily provide large numbers of young.

There is some evidence of differences in the nutritive requirements of rats and mice, the latter requiring, according to Wheeler (1913), a higher concentration of protein and inorganic salts in the diet than the former. Several previous investigators in this laboratory—Judson (1916), Thompson (1917), Mitchell (1921) and Bell (1922)—have studied various phases of the nutrition of mice. The exact nutritive requirements of this species seem to have been little studied in the past. The present investigation affords data upon the normal growth, calorie, protein, inorganic salt and vitamin requirements, together with observations upon the relation of diet to reproduction. Incidentally other features of physiological importance have been observed.

The selection and care of mice. The stock animals, obtained from a breeder in Philadelphia, were fed a mixture of commercial wheat bran and skim milk powder, lettuce and dog biscuit being added three times a week. All experimental animals were bred in the laboratory and were kept in individual cages except in the studies on reproduction. As mice scatter their food considerably at times means had to be devised to eliminate this difficulty as far as possible to permit recording the food intake. A special food receptacle was employed, consisting of an ointment jar with an

[1] The experimental data in this paper are taken from the dissertation submitted by Howard H. Beard in partial fulfilment of the requirement for the Degree of Doctor of Philosophy, Yale University, 1925.

[2] Porter Fellow of the American Physiological Society, 1924–25.

aluminum cover through which was inserted a small aluminum pipe elbow slightly larger than the head of the animal.

Food containers and cages were washed clean twice a week and sterilized with steam once a week. All animals and their food containers were weighed twice a week. Fresh tap water was supplied daily.

The temperature of the animal room was maintained within a range of 75 to 80°F. throughout the course of these experiments except for a few days in the summer when it rose to about 86°F. The daily variation was probably not greater than ± 5°F.

THE NORMAL GROWTH OF THE MOUSE. The "standard" diet fed to

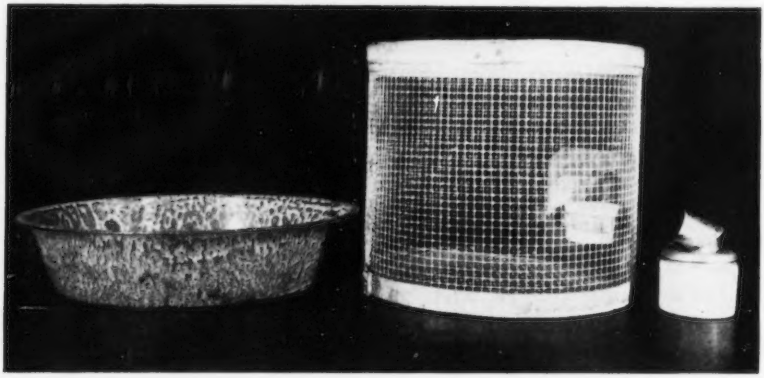

Fig. 1. Showing false bottom cage and special food receptacle.

determine the normal growth of the mouse was based upon that used by Mitchell (1921) and had the following composition:

	grams	*Per cent of total calories*
Casein	31	25
Starch	38	31
"Crisco"	21	39
Salt mixture[3]	7	
Cod liver oil	3	5
Dried yeast (100 mgm. tablet daily)		

This diet furnished approximately 5 calories per gram; but allowing 91 per cent for utilization, 4.6 calories per gram were available.

Twelve male and twelve female mice were placed on the above diet for a period of 27½ weeks after weaning. Chart 1 shows for each sex the curve of average growth based upon body weight. The growth of these animals

[3] Osborne, T. B. and L. B. Mendel. 1919. Journ. Biol. Chem., xxxvii, 572.

was practically the same as that secured with mice by Thompson (1917) and Mitchell (1921) during the first 60 days after weaning. In chart 2 the growth of the above animals is compared with that secured by Robert-

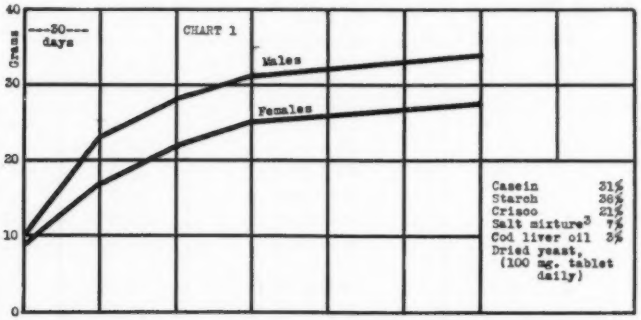

Chart 1. The average growth curves of mice on the "standard" diet.

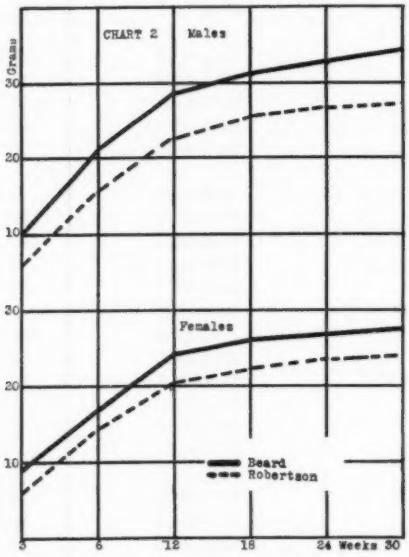

Chart 2. The average growth curves of mice. Comparative data of Robertson and Beard.

son (1916) who fed a diet of rolled barley, the mixed white and yolk of egg, lettuce twice a week and dried bread once a week. The differences in the two growth curves may be due either to the different diets furnished

or to a better strain of animals used in the present investigation. An average adult weight of 27 grams for the females and 34 grams for the males was attained in these studies. The growth here is greater than that re-

TABLE 1

Food intake of normal male mice "standard" food mixture

MOUSE NUMBER	BODY WEIGHT	AVERAGE FOOD INTAKE PER WEEK FOR CORRESPONDING PERIOD	
	grams	*grams*	*cals.*
1	10.0–16.0	13.5	68
	16.0–20.5	14.3	72
	20.5–24.0	16.7	84
	24.0–29.0	21.4	107
	29.0–34.0	23.7	119
4	9.7–16.5	12.5	63
	16.5–21.5	17.3	87
	21.5–24.5	13.5	68
	24.5–29.3	19.0	95
	29.3–34.5	18.0	90
5	11.5–16.5	13.6	68
	16.5–21.5	18.5	93
	21.5–24.8	16.7	84
	24.8–29.3	18.7	94
	29.3–34.0	20.9	105
9	9.0–15.7	19.5	98
	15.7–21.0	18.5	93
	21.0–24.0	20.5	103
	24.0–29.0	18.5	93
	29.0–33.5	19.1	96
16	7.0–15.5	10.0	50
	15.5–21.3	15.4	77
	21.3–24.0	18.0	90
	24.0–29.0	18.9	95
	29.0–34.0	22.8	114
17	7.5–15.5	10.0	50
	15.5–21.3	15.0	75
	21.3–24.5	19.0	95
	24.5–29.5	20.9	105
	29.5–34.0	21.5	108
24	11.0–16.0	9.9	50
	16.0–21.5	10.5	53
	21.5–24.5	17.6	88
	24.5–29.0	12.5	63
	29.0–32.5	13.6	68

ported in any previous study in this connection, as far as the writer is aware.

TABLE 2

Food intake of normal female mice "standard" food mixture

MOUSE NUMBER	BODY WEIGHT	AVERAGE FOOD INTAKE PER WEEK FOR CORRESPONDING PERIOD	
	grams	*grams*	*cals.*
2	11.0–15.3	13.0	65
	15.3–20.3	17.6	88
	20.3–24.5	17.8	89
	24.5–29.2	18.8	94
3	8.3–15.0	15.0	75
	15.0–21.0	17.4	87
	21.0–23.5	19.4	97
	23.5–27.0	19.4	97
10	8.0–15.8	18.2	91
	15.8–20.0	19.8	99
	20.0–24.0	18.6	93
	24.0–27.0	20.0	100
30	9.0–15.0	14.4	72
	15.0–20.5	20.0	100
	20.5–23.5	15.4	77
	23.5–28.0	14.0	70
99	9.0–15.0	12.8	64
	15.0–21.0	17.4	87
	21.0–24.0	18.5	92
	24.0–28.0	19.4	97
101	9.5–15.0	12.0	60
	15.0–20.0	18.0	90
	20.0–24.0	19.4	97
	24.0–27.0	18.4	92
104	9.0–16.0	13.9	70
	16.0–21.0	15.0	75
	21.0–24.0	15.6	78
	24.0–28.0	16.6	83
110	9.0–15.0	14.3	72
	15.0–19.5	15.2	76
	19.5–24.0	13.4	67
	24.0–28.0	16.2	81

THE CALORIE REQUIREMENT. The estimations of the energy requirement were based upon the observed intake of the "standard" diet during

the growth of male and female mice over a period of $27\frac{1}{2}$ weeks after weaning. Tables 1 and 2 show the essential data.

In tables 1 and 2 five periods of growth were selected for the males from 10 to 34 grams, and four periods for the females from 9 to 27 grams. Charts 3 and 4 show the relation between body weight and ingested calories-per-day for the above periods.

Table 3 is an average of tables 1 and 2. The "cal-per-kilo-per-day" item was added in accordance with the procedure of Cowgill (1923) in calculating the diet on a kilo-of-body-weight basis, thereby permitting comparisons with other species (rat and dog) in the vitamin B experiments to follow.

TABLE 3

Energy intake in relation to size "standard" diet

AVERAGE BODY WEIGHT AT BEGINNING AND END OF PERIOD	MEAN	AVERAGE FOOD INTAKE PER WEEK	ENERGY INTAKE PER DAY	ENERGY INTAKE PER-KILO-PER-DAY	$W^{\frac{2}{3}*}$	$\frac{Cal}{W^{\frac{2}{3}}}$
Males						
grams	*grams*	*grams*	*cals.*†	*cals.*		
10.1–15.2	12.6	12.2	9	691	5.4	1.6
15.2–21.1	18.2	15.1	11	593	6.9	1.6
21.1–24.1	22.6	17.0	12	535	8.0	1.5
24.1–29.3	26.7	18.0	13	483	8.9	1.4
29.3–33.9	31.6	19.7	14	446	10.0	1.4
Females						
9.1–15.3	12.2	12.2	9	726	5.4	1.6
15.3–20.4	17.8	17.1	12	685	6.8	1.8
20.4–23.9	22.2	17.0	12	547	7.9	1.5
23.9–27.7	25.8	17.6	13	486	8.6	1.5

* The two-thirds power of the average body weight.

† One gram of food is equivalent to approximately 5 calories.

Rubner (1883) was among the first to make a quantitative study of the influence of size upon metabolism although it was known at that time that smaller animals had, per unit of weight, a greater respiratory exchange than larger ones. When, however, the surface of the animals was considered, a practically constant metabolism per square meter of surface was found for all. The surface of an animal is approximately proportional to the square of a linear dimension while the weight is similarly proportional to the third power of a linear dimension. Accordingly,

$$\text{Surface}^2 = K \times W^3$$

and from an expression of this kind Meeh (1897) derived his formula,

$$S = K \times W^{\frac{2}{3}}$$

where S = surface and W = weight.

If the surface area law be assumed to be valid for mice—and judging from the measurements of heat production reported by Tigerstedt[4], this

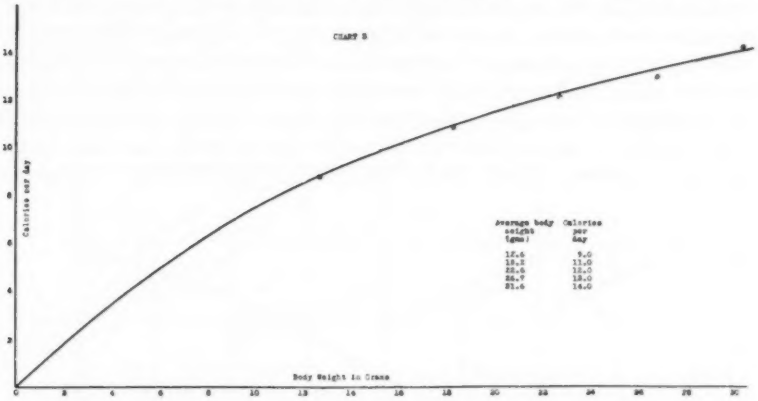

Chart 3. Male mice. Calories-per-day in relation to body weight.

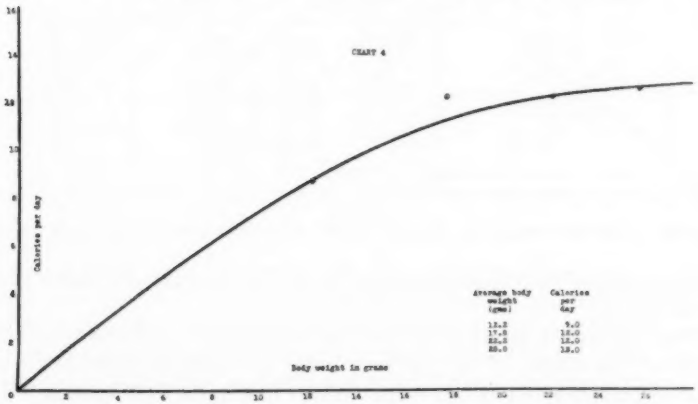

Chart 4. Female mice. Calories-per-day in relation to body weight.

assumption is warranted—calories may be substituted in such a formula for surface. The following expression may, therefore, be formulated:

$$\text{Cal} = K \times W^{\frac{2}{3}}$$

[4] Krogh, A. *The Respiratory Exchange of Animals and Man.* 1916. 141.

This expression has been tested by using data in the literature for dogs, rats and man and has been found to be almost as accurate for determining the basal metabolism of these species as a formula based on surface.[5] Charts 3 and 4 show graphically the results obtained when ingested calories-per-day are plotted against body weight. The curves for the two sexes are almost identical and similar to the customary growth curves obtained when the body weight is plotted against days. Curves of this kind are very definitely logarithmic in character. When, however, the calories-per-day are plotted against the two-thirds power of the weight (chart 5) the relationship becomes more definitely linear. That this relationship is not absolutely linear, however, is readily shown by the fact that a plot on semi-logarithmic paper gives a straight line relationship. The graph

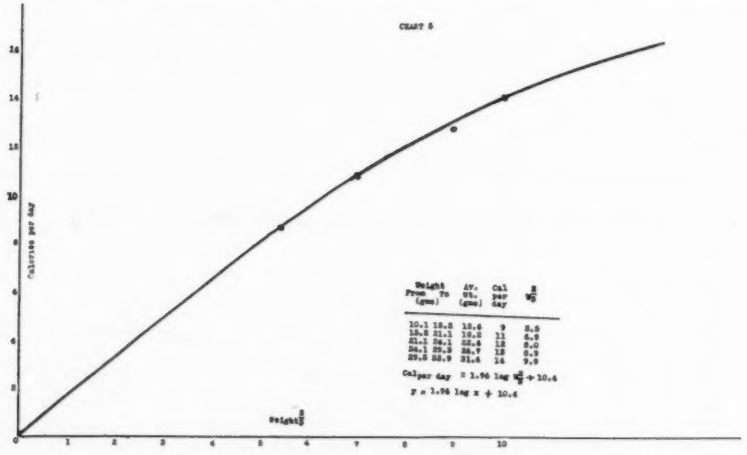

Chart 5. Calories-per-day in relation to the two-thirds power of the body weight.

in this case is of the "slope-intercept" form, the formula of which is given on the chart.

Inasmuch as the relation between calories-per-day and the two-thirds power of the weight of the mouse is only very slightly logarithmic and almost linear, it seems fair to conclude that insofar as their total metabolism is concerned, mice obey the surface area law. Charts 3 and 4 showing the relation of the ingested calories-per-day to the body weight may prove useful in estimating the calorie intake of mice of a given size.

THE PROTEIN REQUIREMENT. The physiology of protein metabolism at the present time, insofar as the rôle of the individual proteins is concerned, resolves itself largely into the question whether or not each will

[5] Personal communication of Dr. G. R. Cowgill.

furnish the indispensable minimum of those amino acids, in physiologically available form, essential for the normal nutrition of an animal. With a more complete knowledge of the amino acid makeup of a protein it has become customary to speak of "complete" and "incomplete" proteins or those of good or poor "biological quality." With the newer methods of investigation which take into account the other known needs of the body—calories, salts, vitamins, etc.—it is possible to make a study of experiments in which the protein becomes the limiting factor in each test.

Wheeler (1913) carried out a number of experiments in feeding different proteins to mice. As far as maintenance was concerned, most of them,

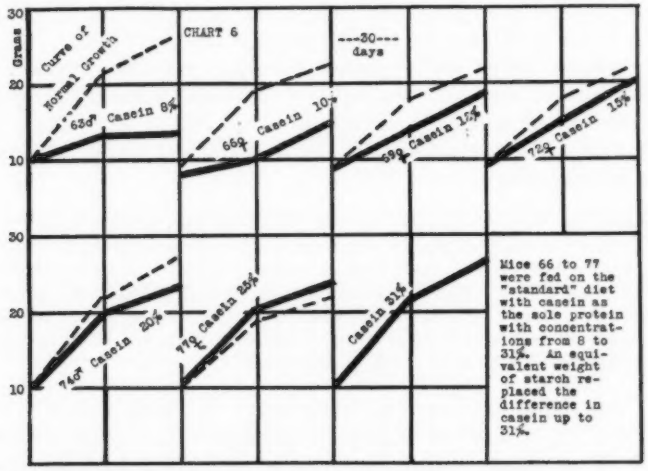

Chart 6. Showing the effects of diets relatively poor in protein on the growth of mice.

excepting gelatin and zein, tested by her were interchangeable. Casein was fed as the sole protein for six months; edestin, gliadin, lactalbumin and glutenin from one to six months. Gelatin or zein employed as the sole protein soon brought about a decline and death of the animals. Of the amino acids, Abderhalden (1922) reported that tryptophane, lysine, arginine, asparagine and glutamic acid are essential for the nutrition of mice.

Experimental. Wheeler (1913) determined that a concentration of at least 31 per cent of protein furnishing 26 per cent of the total ingested calories was required by mice for satisfactory growth under the conditions of her experiment. She fed milk powder, 50 per cent, and casein, 17.3 per cent, giving a total protein content of 30.7 per cent. The writer has confirmed this by feeding casein at various calorie concentrations from 6.5 to

25.1 per cent (8 to 31 per cent casein in the "standard" diet). Chart 6 shows the effects of diets relatively poor in protein on the growth of mice. With a calorie concentration of casein above 9.7 per cent (12 per cent casein) the females made a much better gain in weight than did the males. The best growth was obtained, however, when the casein concentration amounted to 25.1 per cent of the calories (31 per cent of casein in the food mixture). Wheeler stated that mice growing nearly twice as fast as rats apparently need a double proportion of bone- and flesh-forming food substances. It will be shown below that this requisite higher calorie concentration of protein is no doubt due in part at least to the low yield of the essential amino acid, cystine, from casein. When 0.5 gram of cystine was added to each 12 grams of casein in 100 grams of food of unchanged calorie value, growth of the mouse took place at a rapid rate.

THE EFFECT OF FEEDING VARIOUS PROTEINS TO MICE. *Casein.* The results obtained with this protein were discussed above in experiments upon the normal growth of the mouse.

Egg white. Merck's Commercial Egg Albumin was used in these experiments and was coagulated as follows:

> The dry egg white was ground fine and then dissolved in warm water at about 50°C. This syrup was then poured very slowly with constant stirring into 15 liters of water at 90 to 100°C. containing a trace of acetic acid. After coagulation was complete the coagulum was filtered from the solution through cheese cloth, pressed dry as possible in a large hydraulic press, ground in a meat grinder, spread out in large drying pans and desiccated in an oven with a current of warm air. The dried product was then ground fine and incorporated into the diet.

The coagulated egg white replacing casein in the "standard" diet was fed to 7 mice for a period of 90 days after weaning. Chart 7 shows the growth obtained here. There is very little difference between the type of growth secured when either egg white or casein is furnished as the sole protein in the diet.

Gliadin. Wheeler fed this protein to mice and in three months the body weight of some of them did not vary more than a gram from the initial figure. The growth impulse in these cases was only held in abeyance. One of her animals, fed on gliadin as the sole protein for 125 days, made a gain of 5 grams or 50 per cent of its weight when the protein was changed to casein. Judson (1916) fed mice on gliadin food, and stated that when stunting is occasioned by a deficiency of lysine in the diet the results are similar to those produced by reducing the proportion of an adequate protein.

The "standard" diet containing 31 per cent of gliadin in place of an equivalent amount of casein, was fed to four mice. Chart 8 shows the results obtained here. After 26 days on this diet, the gliadin was replaced by an equivalent amount of casein. Resumption of approximately normal growth was rapid in most cases.

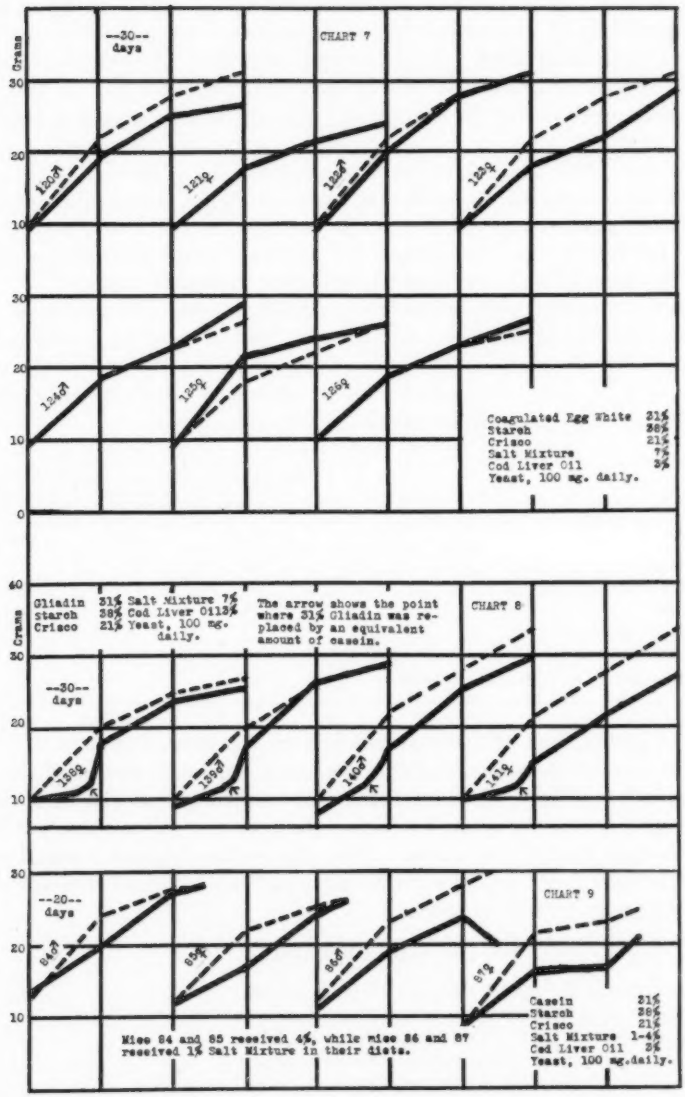

Chart 7. Demonstrating growth of mice on coagulated egg white as the sole protein.
Chart 8. Demonstrating growth of mice on gliadin as the sole protein.
Chart 9. Demonstrating growth of mice on diets poor in inorganic salts.

THE INORGANIC SALT REQUIREMENT. Wheeler (1913) found that a concentration of about 7 per cent of inorganic matter in the diet gave the best growth of her mice. The "protein-free-milk" of Osborne and Mendel (1911b) was used as the source of salts in the above case. Röhmann's salt mixture[6] was also fed but death occurred unless a change in the diet was made.

Judson (1916) made analyses of the entire body of 88 mice at different stages of growth, fed upon different diets, and found among other things a relative increase in the ash content from 1.9 per cent in a newborn mouse to 3 per cent in the adult mouse. A reduction in the content of salts in the diet from 5.7 to 1.7 per cent was sufficient to retard growth.

In the present investigation it was desired to determine the proportion of the Osborne and Mendel salt mixture that is required by the mouse for its optimum growth. Two mice were fed on the "standard" diet containing 4 per cent of this salt mixture while two others received only 1 per cent. The composition of the diets was as follows:

	STANDARD DIET (7 PER CENT SALT MIXTURE)	STANDARD DIET (4 PER CENT SALT MIXTURE)	STANDARD DIET (1 PER CENT SALT MIXTURE)
	grams	*grams*	*grams*
Casein	31	31	31
Starch	38	41	44
"Crisco"	21	21	21
Salt mixture	7	4	1
Cod liver oil	3	3	3
Dried yeast (100 mgm. tablet daily)			

The growth data of these experiments are represented in chart 9. The animals on the lowest concentration of salts were very weak and showed rough coats of hair, while the nails were completely lost in several cases. The condition of the animals receiving the 4 per cent salt mixture was little better than that of the others. From the results obtained it would seem that at least 7 per cent of a "well-balanced" salt mixture is necessary for the optimum growth of mice.

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STUDIES IN THE NUTRITION OF THE WHITE MOUSE[1]

II. The Effect of Feeding Diets Rich in Protein and Diets Containing Unbalanced Salt Mixtures. The Rôle of Certain Sulfur Components

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It has been a widespread belief for a number of years that feeding excessive amounts of protein to animals is detrimental. Polvogt, McCollum and Simmonds (1923), for instance, fed four groups of young rats on diets containing protein varying in amount from 31 to 41 per cent for 400 days. The kidneys of all their experimental animals were reported to show a characteristic picture when contrasted with those of their control rats. The kidneys from animals on the diets rich in protein were large and congested; they showed the formation of hyaline casts, but the glomeruli were little involved.

Osborne and Mendel (1921) on the other hand grew rats at an early age of their experiments to three times their weight on diets containing more than 90 per cent of casein and in which the vitamin-bearing substances were the only noteworthy sources of either fat or carbohydrate, and supplied 4 to 8 per cent of the food eaten. These investigators have recently demonstrated that rats on diets rich in protein show marked hypertrophy of the kidneys. Mendel (1923) states:

> Whether this is due to an inability of the animals to metabolize the quantity of protein required (when it is almost the sole source of energy) beyond a certain structural size, or whether the intake is limited by the limited capacities of even the hypertrophied organs cannot yet be foretold. At present one is probably not justified in making too enveloping deductions as to the effects of protein per se fed with great liberality (p. 131).

Osborne, Mendel, Park and Winternitz (1925) found that the kidneys of rats raised on diets rich in protein became strikingly enlarged, the in-

[1] The experimental data in this paper are taken from the dissertation submitted by Howard H. Beard in partial fulfilment of the requirement for the Degree of Doctor of Philosophy, Yale University, 1925.

[2] Porter Fellow of the American Physiological Society, 1924-25.

crements in size often exceeding 50 per cent of the normal weight of these organs. Preliminary histological examinations failed to disclose changes of an inflammatory or degenerative nature. When a lower percentage of protein was subsequently given the animals the kidneys gradually became normal again. Jackson (1925), in studying the effect of diets rich in protein on the kidneys of rats, found these organs to be considerably hypertrophied but there were no lesions in the glomeruli, tubules or interstitial tissue.

There is some further evidence that the kidneys may not be damaged by excessive protein feeding. Drummond (1922) fed rats and cats on diets containing 80 to 90 per cent casein. The organs and tissues appeared normal and the kidneys were not injured by the excretion of very large amounts of nitrogenous waste products.

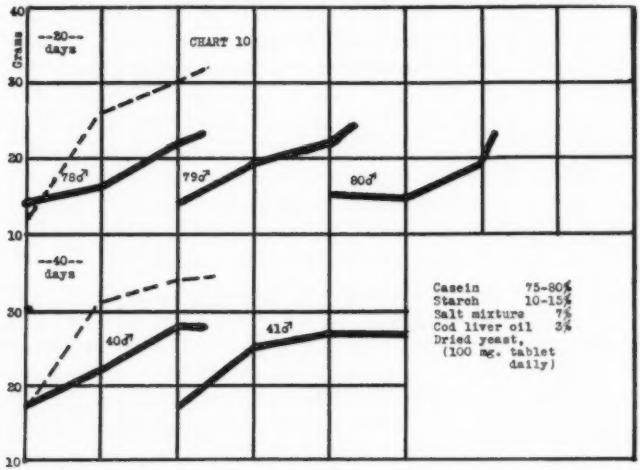


Chart 10. Demonstrating growth of mice on diets rich in protein.

Experimental. In the present experiments with diets rich in protein the food mixture consisted of:

	grams
Casein	75–80
Starch	10–15
Salt mixture[3]	7
Cod liver oil	3
Dried yeast (100 mgm. tablet daily)	

Chart 10 shows that the rate of growth was subnormal when mice were fed the above diet. The weights of several organs, including the kidneys,

[3] Osborne, T. B. and L. B. Mendel. 1919. Journ. Biol. Chem., xxxvii, 572.

are given in table 4 in comparison with data from mice on the "standard" diet containing 31 per cent of casein.

In group A the animals were permitted to reach 15 grams body weight. One lot was fed on the "standard" diet with 18 per cent urea replacing an equivalent amount of starch, while the other lot was fed 80 per cent of casein. A comparison of the organ weights of these animals with those of the controls shows little difference in weight.

TABLE 4

Comparative weights of organs of mice on the "standard" food mixture and on diets rich in protein

GROUP	MOUSE NUMBER	BODY WEIGHT	DURATION OF FEEDING TEST	DIET	HEART	LIVER	KIDNEYS	
							Right	Left
		grams	*days*		*gram*	*gram*	*gram*	*gram*
A	127	15.0	27	"Standard" 31 per cent casein	0.10	1.05	0.14	0.13
	128	15.0	28		0.11	1.07	0.18	0.16
	129	15.3	30		0.11	1.27	0.17	0.17
	131	15.0	28	80 per cent casein	0.10	1.23	0.14	0.14
	132	15.3	32		0.11	1.24	0.16	0.19
	133	15.0	46	"Standard" + 18 per cent urea	0.10	1.02	0.18	0.16
	134	15.0	57		0.09	1.28	0.17	0 17
	135	15.2	53		0 10	1.18	0.18	0.19
B	105	29.0	50	"Standard" 31 per cent casein	0.18	2.11	0.34	0.33
	106	26.0	50		0 14	1 98	0 31	0.31
	107	25.5	50		0.16	2.02	0.32	0.32
	78	22.0	50	75 per cent casein	0.14	1.63	0.26	0.26
	79	23.0	50		0.14	1.84	0.29	0.29
	80	22.0	50		0.14	1.27	0.28	0.28
	81	19.5	50		0.14	1.32	0.28	0.28
C	43	33.0	107	"Standard" 31 per cent casein	0.21	2.43	0.35	0.35
	44	32.0	107		0.22	1.74	0.41	0.41
	45	31.0	107		0.20	2.08	0.45	0.43
	40	25.5	107	80 per cent casein	0.21	1.97	0.40	0.33
	41	27.5	107		0.19	1.97	0.39	0.40
	42	26.0	107		0.16	1.23	0.95	0.85

The animals of group B were on the diet rich in protein for 50 days. In this group the livers weighed slightly less than the livers of the control animals, while the kidneys of the former were slightly less than the kidneys of the latter, respectively.

In group C the animals were fed the diet rich in protein for 107 days. The differences in weight here of the liver and kidneys, when compared

with those of the control group, while small, are somewhat more striking. There is about the same difference in weight of the livers in this group as in group B. The average weights of the kidneys of the experimental animals are about 0.17 gram greater than those of the controls. This may be due to the fact that the experimental animals were on the diet rich in protein for a much longer time than those of the former groups. These differences in weight, being small, show that the effect of feeding diets rich in protein as here undertaken is probably without noteworthy effect on the weight of the kidneys.

THE EFFECT OF FEEDING UNBALANCED SALT MIXTURES. It is a well-established fact that the animal body requires calcium and phosphorus for the normal metabolism of its skeletal tissues. Green (1921) stated that if the ration of cattle be deficient in phosphorus a condition called osteophagia appears which is common in parts of South Africa most of the year and has been traced to a phosphorus deficiency in the soil and vegetation. This disease may also be produced experimentally in cattle by feeding diets poor in phosphorus.

A lack of either calcium or phosphorus or both has been shown under certain conditions by several investigators to cause rickets in rats. A distinct advance was made in the experimental production of this disease by McCollum, Simmonds, Shipley and Park (1921) and also by Sherman and Pappenheimer (1921) who used diets deficient in mineral substances. The former investigators reported that rats fed the deficient diets developed abnormal conditions in the skeleton very closely resembling human rickets. All of the diets used were deficient in fat soluble vitamin, while one lacked calcium and another lacked phosphorus. The results were interpreted as demonstrating the existence of two forms of rickets, one characterized by a low Ca:P and the other by a high Ca:P ratio in the blood.

Sherman and Pappenheimer demonstrated the presence of rickets in rats by means of a diet low in phosphorus (diet 84) and its prevention by addition of alkaline potassium phosphate (diet 85) to the food. The former diet was not only deficient with respect to vitamins A, B and C, but was poor in protein, potassium and phosphate. It was difficult therefore to attribute the rachitic condition to a lack of phosphorus only. However, Pappenheimer, Zucker and Hess (1922a, b) showed that the addition to this diet of yeast, orange juice, butter fat and potassium did not prevent its rickets-producing power.

Another advance in this line of investigation was made by Osborne, Mendel and Park (1923) who studied the production of experimental rickets in rats under more definitely controlled dietary conditions. They employed a diet in which one component of the salt mixture could be varied at a time. In these studies they used the same calcium- and phosphorus-free salt mixtures as had been employed in a previous study (Osborne and

Mendel, 1918). Their rats were placed on a diet lacking either calcium or phosphorus for 35 days, killed and examined, marked rickets appearing as the gross and microscopic changes were characteristic of this disease.

Experimental. In studying the rôle of some of the inorganic elements in nutrition of mice six animals were fed on the Sherman-Pappenheimer diets 84 and 85. After 120 days on these diets the animals were killed and gross examination of the ribs and femur did not reveal any rachitic changes. It is possible that the length of time these animals were fed on these diets might have been long enough for spontaneous cures to have taken place.

In the next series of experiments the procedure of Osborne, Mendel and Park (1923) was followed. Edestin was employed, thus avoiding the use of a phosphorized protein; no fat was added to the food mixture as rickets may not develop well if this foodstuff be present. During growth all animals were kept in a dark room for a period of 28 days after weaning in the belief that darkness would hasten the onset of rickets under the conditions laid down.

The control group received the "standard" diet; a second group received the diet below which contained a phosphorus-free salt mixture,[4] while a third group received the same diet but with a calcium-free salt mixture.[5] Chart 11 shows the growth data of these experiments. The composition of the experimental diets was as follows:

Phosphorus-free diet	*grams*		*Calcium-free diet* *grams*
Edestin	31.00		31.00
Starch	60.85		60.85
Salt mixture[4] (P-free)	7.00	Salt mixture[5] (Ca-free)	7.00
Na_2HPO_4	1.15		1.15
Dried yeast (100 mgm. tablet daily)			

Results. Histological examinations revealed an essentially normal bone for all groups mentioned above. With calcium present (phosphorus absent) in the salt mixture growth was better than when phosphorus was present (calcium absent). The growth of both the second and third groups when compared with that of the controls was subnormal.

Conclusions. Mice were fed on two types of diets (Sherman-Pappenheimer, and Osborne, Mendel and Park) to determine if rickets could be produced experimentally in these animals as has been claimed for rats under similar conditions. Gross and histological examinations of the bones did not reveal any rachitic changes in these cases. Calcification of the bones was essentially normal.

THE RÔLE OF CERTAIN SULFUR COMPONENTS. *Can taurine replace cystine in the diet of mice?* The importance of cystine for the normal growth

[4] Osborne, T. B. and L. B. Mendel. Journ. Biol. Chem., 1918, xxxiv, 121.
[5] Osborne, T. B. and L. B. Mendel. Journ. Biol. Chem., 1918, xxxiv, 121.

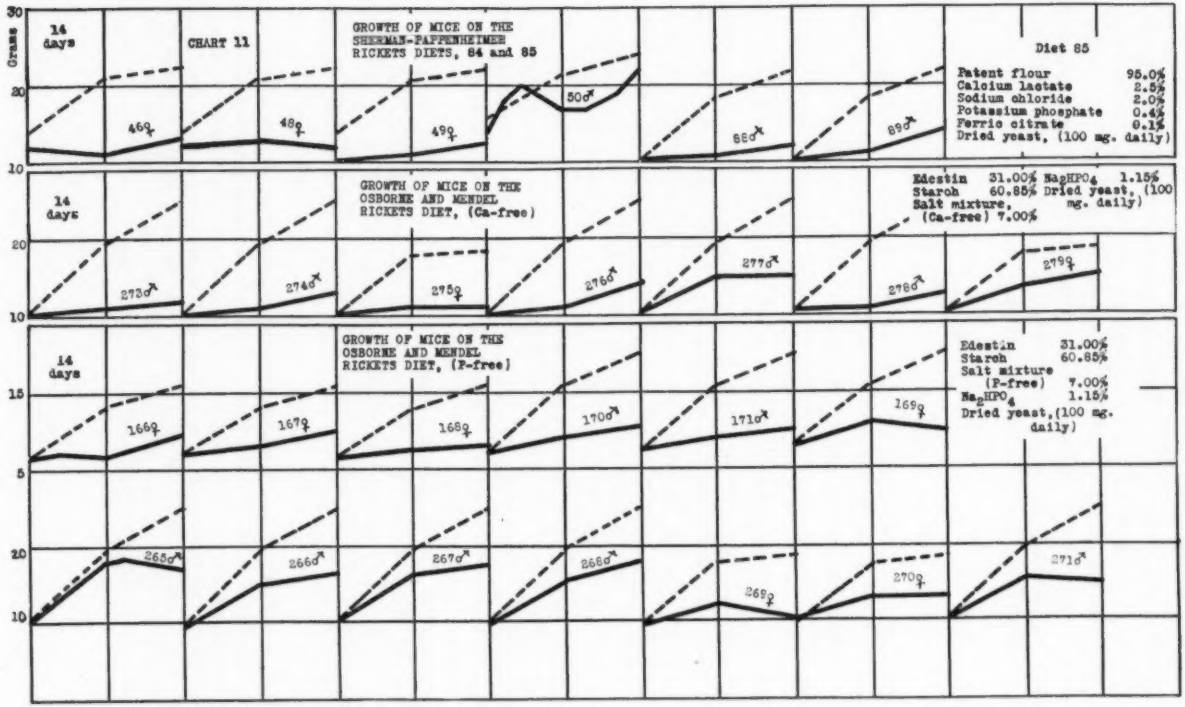
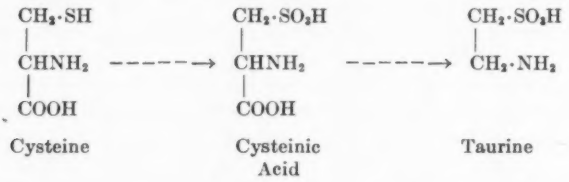


Chart 11. Demonstrating growth of mice on diets containing unbalanced salt mixtures.

of young white rats has been shown by Osborne and Mendel (1915) and also by Johns and Finks (1920). Neither elementary sulfur (Geiling, 1917) nor sulfates (Daniels and Rich, 1918) are able to serve as a source of sulfur and cystine in supplying the requirements of rats and mice. Cystine is supposed to give rise to taurine in the animal body, although the mechanism by which this occurs is unknown. The oxidation of cystine or cysteine is supposed to take place as follows:



Cysteine — Cysteinic Acid — Taurine

Mitchell (1924) published observations which he believed indicated that taurine could replace cystine in the diet of mice. This investigator used three sets of animals; the first, serving as controls, received 15 per cent casein in the diet, and the other two sets received a diet deficient in cystine, (casein 9 per cent), to which this amino acid and taurine respectively were added at various intervals. Better growth was secured in the case of the addition of taurine than that of cystine. Mitchell accounted for this result by assuming that the diet might have been adequate in either case because it contained some factor common to both taurine and cystine, namely, sulfur. He stated that his taurine might have contained traces of cystine, and if this is so the beneficial effects of the former might have been due to this impurity.

Experimental. In the present investigation it was desired to repeat the work of Mitchell indicative that taurine can replace cystine in the diet of mice. Cystine for feeding was prepared by the writer by acid hydrolysis of hair; taurine was prepared from fresh ox bile.[6] The composition of the diets fed was as follows:

[6] Fresh ox bile was boiled for some hours with dilute hydrochloric acid and the filtrate from the insoluble anhydrides of the bile acids was concentrated on the water bath to a small volume and filtered while hot from sodium chloride. The solution was then evaporated to dryness and the residue dissolved in 5 per cent hydrochloric acid. Taurine was then precipitated from solution by the addition of 10 volumes of 95 per cent alcohol. The crystals were then purified by solution in acid, precipitation by alcohol and recrystallization from water.

	DIET 1 (CONTROL)	DIET 2 (CYSTINE)	DIET 3 (TAURINE)
	grams	*grams*	*grams*
Casein	12.0	12.0	12.0
Cystine	0.0	0.5	0.0
Taurine	0.0	0.0	0.5
Starch	57.0	56.5	56.5
Salt mixture[7]	7.0	7.0	7.0
"Crisco"	21.0	21.0	21.0
Cod liver oil	3.0	3.0	3.0
Dried yeast (100 mgm. tablet daily)			

Three groups of animals were used. The first group, serving as controls, received the "standard" diet (diet 1) containing 12 per cent of casein. Previous investigations had demonstrated (chart 6) that this concentration of casein was insufficient for the maximum growth of mice. The growth data are given in chart 12.

The second group received the same food as the first with the addition of 0.5 gram of cystine to the casein (diet 2). Satisfactory growth was obtained with it for a period of 28 days after weaning, at which time this group was given the same diet but with an equivalent amount of taurine replacing the cystine (diet 3). A slight decline in weight resulted.

The third group was fed diet 3, containing 0.5 gram of taurine, from the beginning. The growth curves for this group and that of the controls on 12 per cent casein are almost identical for the first 14-day period after weaning. At this time this group was given diet 2, containing cystine instead of taurine, and resumption of growth was rapid in all cases.

CONCLUSIONS.

The results here obtained do not confirm the conclusion of Mitchell (1924) that taurine can replace cystine in the diet of mice. It is also shown, insofar as taurine and cystine are concerned, that it is not merely "sulfur" that is required by the organism, but "cystine-sulfur."

These experiments also demonstrate that cystine is the limiting amino acid factor in casein when this protein is fed at a concentration of 12 per cent in the diet of mice. The addition of 0.5 per cent of cystine thereto causes growth to take place at a rapid rate. As the cystine-yielding capacity of casein is very low, this is probably one of the reasons why a higher concentration of this protein must be fed to mice to insure their maximum growth.

[7] See footnote 3, page 659.

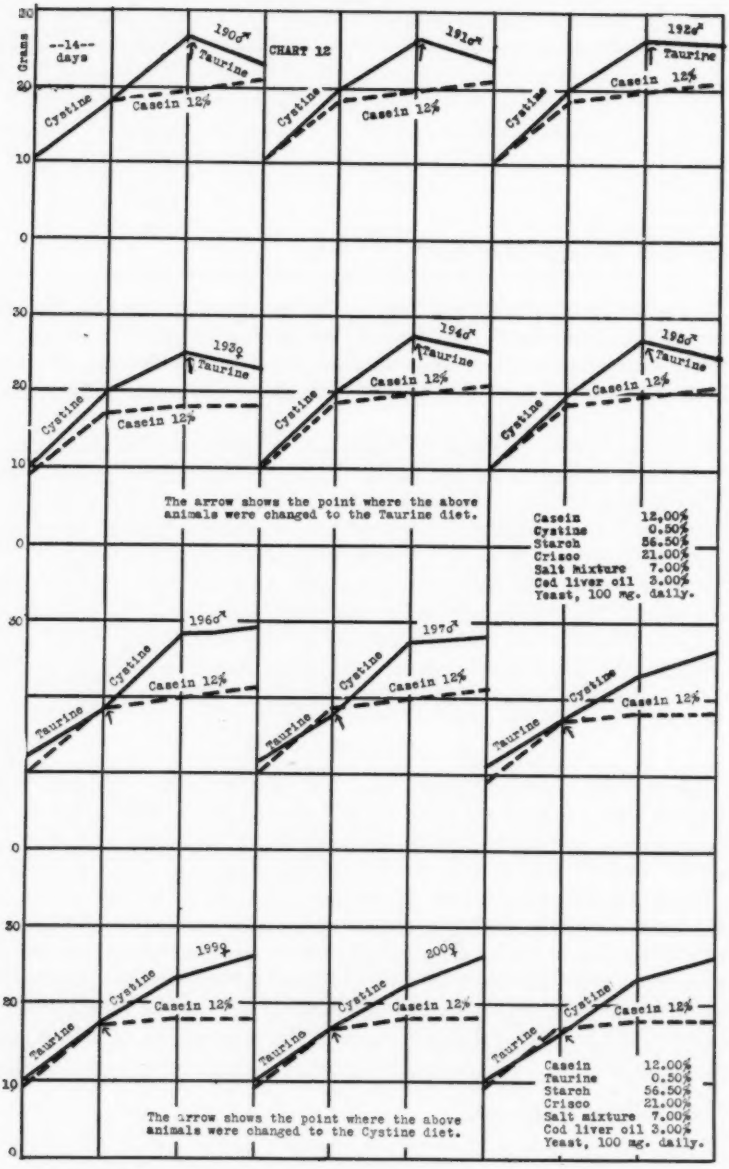


Chart 12. Comparison of taurine and cystine on the growth of mice.

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STUDIES IN THE NUTRITION OF THE WHITE MOUSE[1]

III. Vitamin Factors in the Nutrition of Mice

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It has long been known that a diet adequate in respect to its protein, fat, carbohydrate and salt content may still be unable to support maintenance and growth. The white mouse has been the experimental animal used in most of the earlier experiments in vitamin research. Stepp (1909, 1911a, b; 1913) used these animals in his study of the physiology of the so-called accessory food factors. He fed materials extracted with alcohol and ether and observed the effect on the nutritive equilibrium of his animals. As death occurred in a few weeks upon his diets, Stepp concluded that the failure was due to a lack of certain "lipods" from the diet, because the addition of the ether-alcohol extracts of materials known to be rich in this type of compound sufficed to keep the animals alive. Cooper (1914) suggested that the deleterious effect of the lipod-free diets observed by Stepp was due not to the deficiency of lipods but to the mechanical removal of the vitamin content during the extraction of his food materials.

There have been numerous contributions to the physiology of these unknown substances in recent years. Groebbles (1922), for instance, discussed the relation between avitaminosis and inanition. He believed that in the former condition the heat regulatory mechanism was impaired. In his second paper, involving experiments on mice, he stated that vitamins were metabolism regulators. In some further investigations (1923–24) this investigator studied the action of yeast and oats on the oxygen consumption, body weight and longevity of mice in avitaminosis. In the latter paper he reported experiments on mice with the addition alone and in combination, of butter, yeast and lemon juice, to vitamin-free rice flour. The effect of the complexes A, B and C, on the oxygen consumption, body weight and longevity, did not correspond to the sum of the single effects of these factors. Groebbles concluded from this that the vitamin factors are

[1] The experimental data in this paper are taken from the dissertation submitted by Howard H. Beard in partial fulfilment of the requirement for the Degree of Doctor of Philosophy, Yale University, 1925.

[2] Porter Fellow of the American Physiological Society, 1924–25.

mutually influenced by a sort of synergic effect, and that all three are necessary for the normal growth of the mouse.

In addition to being essential for normal growth, vitamin A, in most species of animals, also acts as an antixerophthalmic substance. The investigation of this phase of the subject as far as mice are concerned seems to have been little studied in the past. Spinka (1923) reported investigations upon the action of ultraviolet rays on these accessory food substances. Mice fed with food containing butter which had been subjected to the action of these rays all perished in 7 to 9 days, owing to the alleged production of substances of aldehydic or ketonic nature, while those fed with food not containing vitamin A lived for about 50 days, dying after having been attacked by keratomalacia.

Bertrand and Benzon (1924) described an investigation upon the importance of zinc in the nutrition of mice. One of their animals lived on a vitamin- and zinc-free diet for 11 weeks and suddenly developed xerophthalmia and died at the end of that time. This is the only incidence of this disease in all their experiments. This case, and those of keratomalacia reported by Spinka above, are the only references to this condition in mice in the literature with which the writer is familiar.

EXPERIMENTAL. *The effect of feeding a diet deficient in vitamin A.* In the present investigations of feeding mice on vitamin-A-free diets, the proteins (casein or coagulated egg white) were carefully extracted twice with hot alcohol and ether in a Shohl-Koch extractor and then dried in a current of air at 120°C. for 48 hours or more to destroy the vitamin. The diets used were as follows:

	grams
Diet 1	
Extracted casein	31
Diet 2	
Extracted casein (Harris)*	31
Diet 3	
Coagulated and extracted egg white	31
Starch	38
"Crisco"	24
Salt mixture[3]	7
Dried yeast (100 mgm. tablet daily)	

* Prepared by the Harris Laboratories, Tuckahoe, N. Y.

Twenty-five mice, divided into three groups, were used. In the first group 8 animals were placed on the extracted casein diet (diet 1). Chart 13 shows the growth data of this investigation. After 100 days upon this food, 3 per cent cod liver oil replaced an equivalent amount of crisco in the

[3] Osborne, T. B. and L. B. Mendel. 1919. Journ. Biol. Chem., xxxvii, 572.

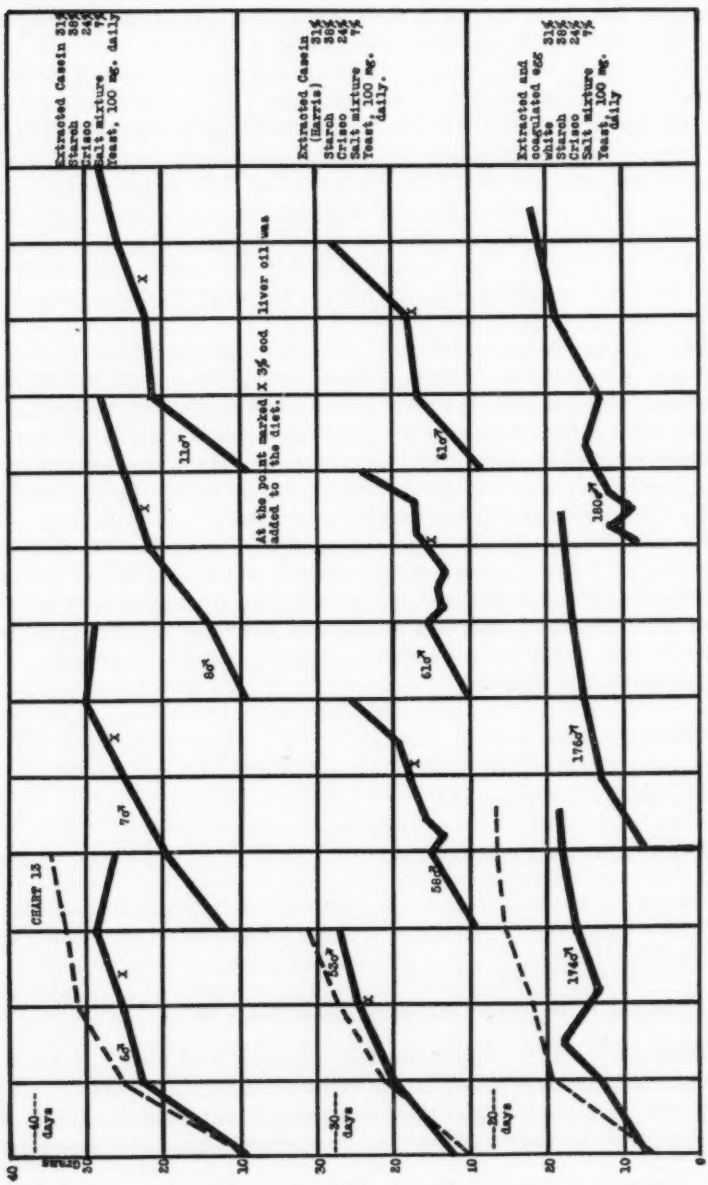

Chart 13. Demonstrating growth of mice on vitamin-A-free diets.

diet, whereupon an increased growth was obtained. Examination of the eyes of a number of these animals failed to reveal any evidence of a pathological condition similar to that found in rats when the latter are fed on a vitamin-A-free diet.

In the second group, 9 animals were fed upon a diet free from vitamin A and containing extracted casein (Harris) (diet 2), for a period of 90 days after weaning. The growth was again checked but was increased when cod liver oil was added to the diet. No ocular manifestations were observed in this group.

In the third group 8 mice were fed upon diet 3, containing coagulated and extracted egg white as the protein. This was coagulated as described above in the protein experiments, then extracted with alcohol and ether just as the casein in the first series of vitamin A experiments above. These animals were kept in a dark room during the course of the experiments for a period of 90 days after weaning. This procedure was adopted as it was desired to study the effect of darkness upon the possible production of xerophthalmia in mice when fed upon a vitamin-A-free diet. The growth was comparable to that of the second group, and again no xerophthalmia was observed.

During the course of the above experiments it was desired to test out each experimental diet on the rat, to determine if the food would produce the characteristic eye disease in this species. One rat for each of the above groups was fed the same diet as the experimental mice, but with a concentration of 18 per cent extracted protein. In each case the rat developed xerophthalmia, which was cured in 7 to 10 days by the addition of cod liver oil to its diet. Therefore it was clearly shown that all three diets were free from vitamin A as shown by the rat-test method.

Conclusions. On the basis of the above results we may conclude that vitamin A is necessary for the normal growth of the mouse, but that in its absence the eye disease, xerophthalmia, does not readily appear in the characteristic way so often found in rats and some other species, when the diet is free from this dietary essential.

The effect of feeding a diet deficient in vitamin B. All species of animals so far studied have been found to require vitamin B for their nutritive well being. Comparatively little is known however concerning the precise function of this accessory factor. It is undoubtedly essential for maintenance of appetite and growth and it acts to prevent the appearance of nervous and muscular symptoms characteristic of the syndrome commonly spoken of as polyneuritis. Practically all students of vitamin B function agree on the facts just stated.

In the literature concerning the physiological rôle of this accessory factor two different views in addition to the above are held: 1, vitamin B is something required by all cells of the animal body and it plays some funda-

mental rôle in the physiological activity of same, and 2, it is not required by all cells, but acts physiologically to stimulate certain organs to activity. The work of Abderhalden and his collaborators (1920; 1921a, b, c) may be cited in support of the first view, whereas Cramer (1923) and his associates are proponents of the latter view.

The experiments of Abderhalden seem to show that the tissues of polyneuritic birds possess a lower oxidative capacity than that of normal birds. This condition is not limited to one type of tissue but appears to be quite general and is more satisfactorily explained by the assumption that vitamin B functions in some way in the physiological activity of *all* cells. The evidence cited by Cramer in support of his "food harmone" hypothesis is based upon blood cell counts made in normal rats and those suffering from avitaminosis. He advances the theory that vitamin B acts to stimulate lymphoid tissue. In distinction from Cramer (1923), Uhlmann (1918) and Voegtlin and Myers (1919), Cowgill and Mendel (1921) did not find any direct relation between vitamin B and the secretory function of the pancreas, liver and salivary glands.

Most of the studies on vitamin B phenomena have been carried out in a qualitative manner. Osborne and Mendel (1922) pointed out the quantitative relationship existing between vitamin B and the growth of the rat. They added various amounts of this vitamin in the form of dry brewery yeast to a diet otherwise free from this factor. Their results were believed to indicate that the vitamin B requirement of rats for maintenance and growth bears a quantitative relationship to the mass of "active tissue." Under the conditions which they maintained 100 grams of body weight of rat required from 50 to 60 mgm. of dry brewery yeast per day for adequate growth. Recently they used yeast concentrate (1925) and found that the minimum effective dose of same that is required to produce growth at approximately normal rate may be placed at 15 to 29 mgm. per 100 grams of body weight in contrast with 50 mgm. of the yeast itself.

These results of Osborne and Mendel are suggestive, and, when taken in conjunction with the quantitative data obtained in this laboratory by Cowgill, Deuel and Smith (1924) and Cowgill, Smith and Beard (1925) point to a possible relationship between the vitamin B requirement, the size of the animal, and metabolic rate. Experiments were, therefore, conducted on the mouse in an endeavor to determine more precisely its daily requirement of a substance containing vitamin B.

EXPERIMENTAL. In the first series of experiments the animals were fed the "standard" diet, the casein of which had been previously extracted with hot alcohol to free it from vitamin B. Various daily amounts of Yeast Vitamin Powder (Harris) were supplied apart from the diet. The composition of the basal diet fed in all the vitamin experiments reported below had the following composition:

	grams
Extracted casein	31
Starch	38
"Crisco"	21
Salt mixture[4]	7
Cod liver oil	3

Yeast vitamin powder (Harris)* 10–50 mgm. daily

* Supplied by the Harris Laboratories, Tuckahoe, N. Y.

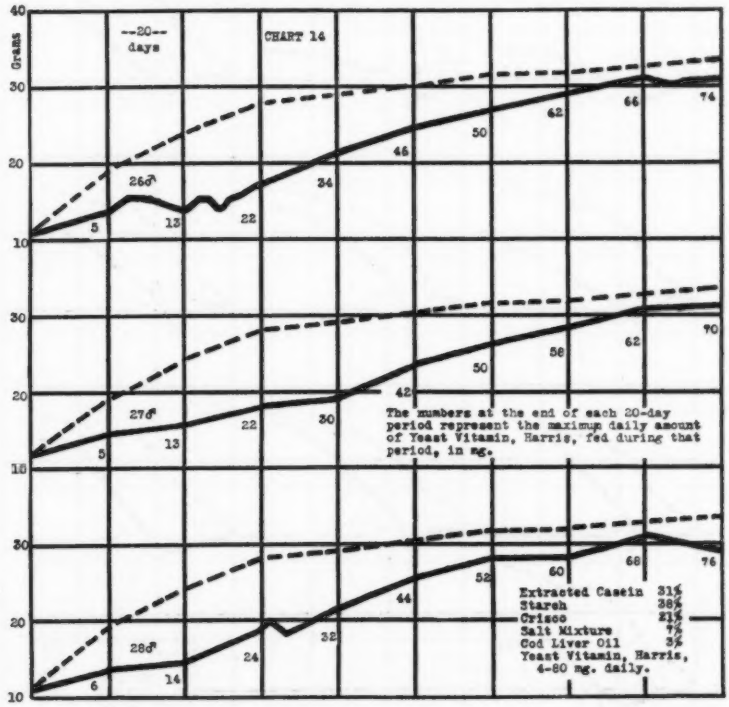

Chart 14. Demonstrating growth of mice with daily additions of Harris' yeast vitamin. Quantitative experiments.

In the first series of experiments (chart 14) the effects of the various vitamin doses on the growth rate were not pronounced, perhaps because the vitamin increments were not large enough. The animals eventually reached adult size, growth proceeding slowly. On the basis of the data thus obtained it became difficult to decide precisely what vitamin dosage was required for any given body weight. However, in this series the effect

[4] See footnote 3, p. 669.

of a suboptimal dose of vitamin B on the growth rate of the mouse is apparent. This is similar to the result obtained by Osborne and Mendel with the rat when they used doses of 25 and 50 milligrams of dry brewery yeast per day and the smaller doses of yeast concentrate.

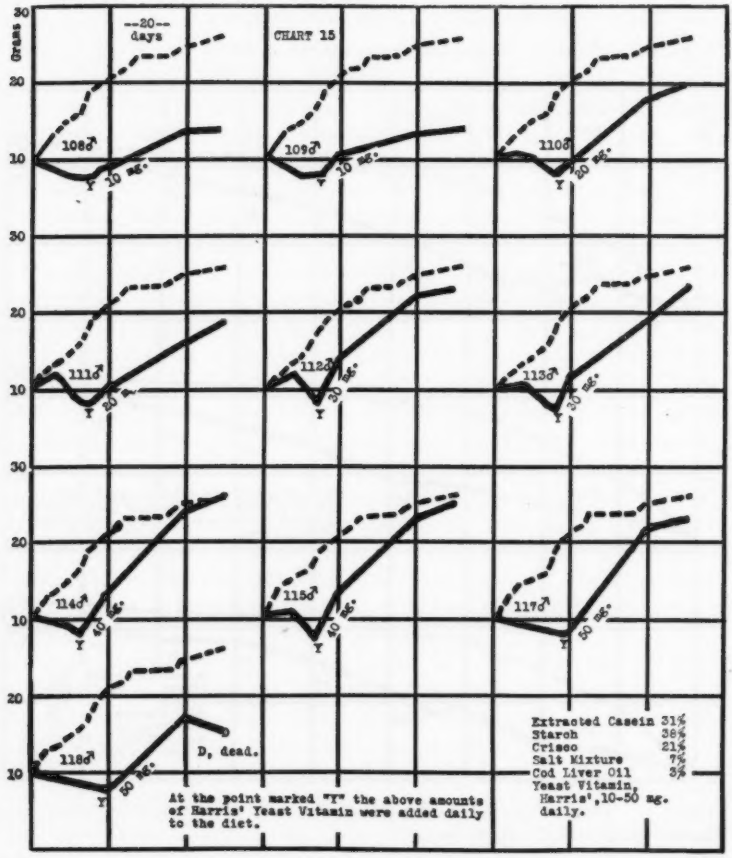

Chart 15. Demonstrating growth of mice with daily additions of Harris' yeast vitamin. Quantitative experiments.

In the second series (chart 15) the animals were first given the vitamin-B-free diet for 18 days; then various doses of this vitamin from 10 to 50 mgm. daily were fed apart from the diet. The behavior of the experimental animals in this connection is exactly like that of rats, when the latter are fed a diet free from vitamin B. For a short period the animals

grow, then a loss of body weight occurs, and the administration of vitamin B to an animal that has thus declined brings about a remarkable recovery. *Forty milligrams* of the Yeast Vitamin Powder (Harris) proved to be the minimum amount capable of allowing maximum growth in animals that had suffered from vitamin B deficiency. In experiments of this type any

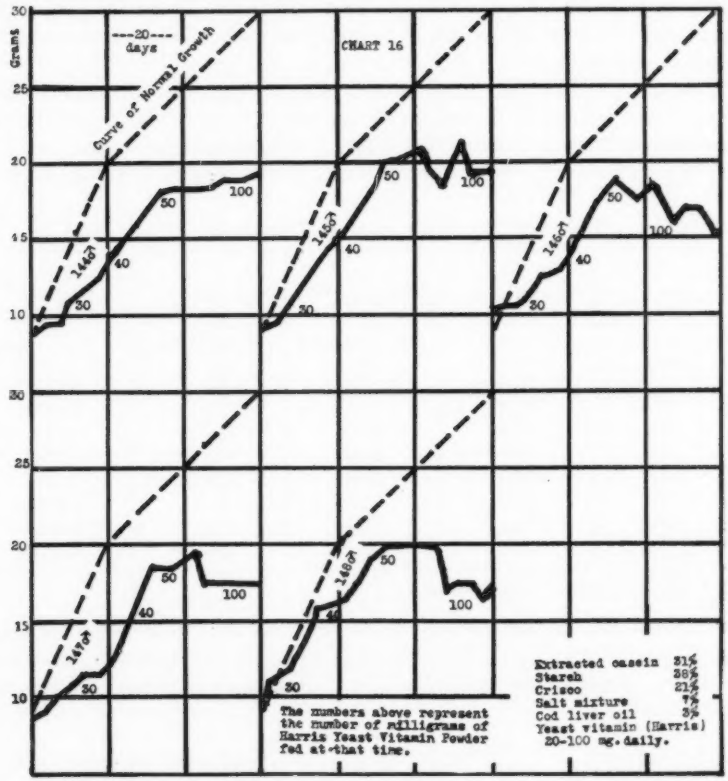

Chart 16. Demonstrating growth of mice with daily additions of Harris' yeast vitamin. Quantitative experiments.

possible store of this accessory factor in the tissues is depleted before the material to be tested is given.

In the third series of quantitative experiments (chart 16) the plan followed by Osborne and Mendel (1925) was adopted. Six male mice, started at 10 grams body weight, received 20 mgm. of the Yeast Vitamin Powder (Harris) per day. This dose was then increased at intervals and 40 mgm. again proved to be the minimum daily amount necessary for

growth up to 18 grams body weight. Increments up to 100 mgm. daily failed to give any better growth. No reason can be given at the present time for this failure to grow after the animals had reached the body weight mentioned above. It was thought that the physical texture of the diet may have been at fault as the animals would invariably scatter their food; however when the unextracted "standard" diet was given, they still scattered it just the same as the vitamin-B-free diet. This question will be studied at a later date.

Discussion. Cowgill, Smith and Beard (1925) published results, based upon data obtained with the dog, rat and mouse, by which it was shown that the vitamin B requirement of these species of animals is closely proportional to the product of: 1, the two-thirds power of the weight; 2, the quantity of

TABLE 5

The vitamin constant of the mouse

VITAMIN PER DAY*	BODY WEIGHT	ENERGY INTAKE PER DAY	$\text{Cal} \times \text{W}\frac{5}{3}$	$\frac{\text{VITAMIN}}{\text{Cal} \times \text{W}\frac{5}{3}}$ (K)
mgm.	*grams*	*cals.*		
10	14.2	9.0	77	0.0126
10	14.0	9.0	75	0.0134
20	20.0	11.0	165	0.0121
20	19.0	11.0	148	0.0133
30	23.0	12.0	225	0.0133
30	23.0	12.0	225	0.0133
40	26.0	13.0	294	0.0136
40	26.0	13.0	294	0.0136

* Yeast Vitamin Powder (Harris).

energy metabolized as indicated by the total calories utilized from the food; and 3, the body weight. The following formula was proposed to show these factors. By it a number called the "vitamin B constant" K may be calculated,

$$K_{\text{vitamin}} = \frac{\text{vitamin}}{\text{calories} \times \text{weight}^{\frac{5}{3}}}$$

Using the data obtained with mice in the second series of experiments above, and applying this formula we get the results tabulated in table 5.

Table 6 (chart 17) compares the above results with those of other species, namely, the rat and dog. The value of K, the vitamin constant, is greatest in the case of the mouse and smallest in case of the dog.

Summary and conclusions. The methods of vitamin B testing that have been developed for the rat and dog can also be used advantageously with the mouse.

The mouse, like other species studied, requires this vitamin for its normal nutrition.

Forty milligrams of Yeast Vitamin Powder (Harris) daily were found to be adequate for growth up to a body weight of 18 grams. Satisfactory growth above this point, using a highly purified diet, was not obtained. An explanation of this at present is not at hand.

Quantitative experiments show that the requirement of the mouse for vitamin B is in linear relationship to the product of: 1, its weight; 2, the number of calories metabolized per day, and 3, its surface. A similar relationship has been found for the rat and dog by Cowgill, Smith and Beard (1925).

TABLE 6

*Comparison of the vitamin constant of the mouse, rat and dog**

SPECIES	VITAMIN PER DAY†	BODY WEIGHT	ENERGY INTAKE PER DAY	$\frac{\text{VITAMIN}}{\text{Cal} \times \text{W}^{\frac{5}{3}}}$ (K)
	mgm.	*grams*	*cals.*	
Mouse	10	14.1	9	0.013
	20	19.5	11	0.013
	30	23.0	12	0.013
	40	26.0	13	0.014
	(Vitamin = 0.0135 Cal $\times$ W $^{\frac{5}{3}}$ $-$ 0.5)			
Rat	20	97.2	25	0.000,38
	30	129.0	32	0.000,29
	45	153.0	39	0.000,27
	(Vitamin = 0.000,217 Cal $\times$ W $^{\frac{5}{3}}$ + 7.8)			
Dog	90	4550.0	383	0.000,000,19
	165	5800.0	383	0.000,000,18
	232	8000.0	495	0.000,000,14
	360	9700.0	515	0.000,000,15
	(Vitamin = 0.000,000,19 Cal $\times$ W $^{\frac{5}{3}}$ $-$ 7.2)			

* The data for the rat and dog in this table are used for comparison with the permission of Dr. G. R. Cowgill from some of his unpublished data on this subject.

† Yeast Vitamin Powder (Harris).

These studies indicate that vitamin B acts as a "building stone" in metabolism and is required in the physiological activity of all cells of the body.

The effect of adding vitamin C to the "standard" diet. It is probable that the rat and dog do not require vitamin C for their normal growth. Cowgill (1925) has kept a dog over a long period of time without any of this vitamin in its diet. Groebbles (1923–24) fed all three vitamins, A, B and C separately and then in different combinations, and stated that the best growth of mice was obtained when all three were included in the diet. Tönnis (1924) also fed different combinations of these accessory factors to mice. His curves show that with a combination of any two, there was a decline

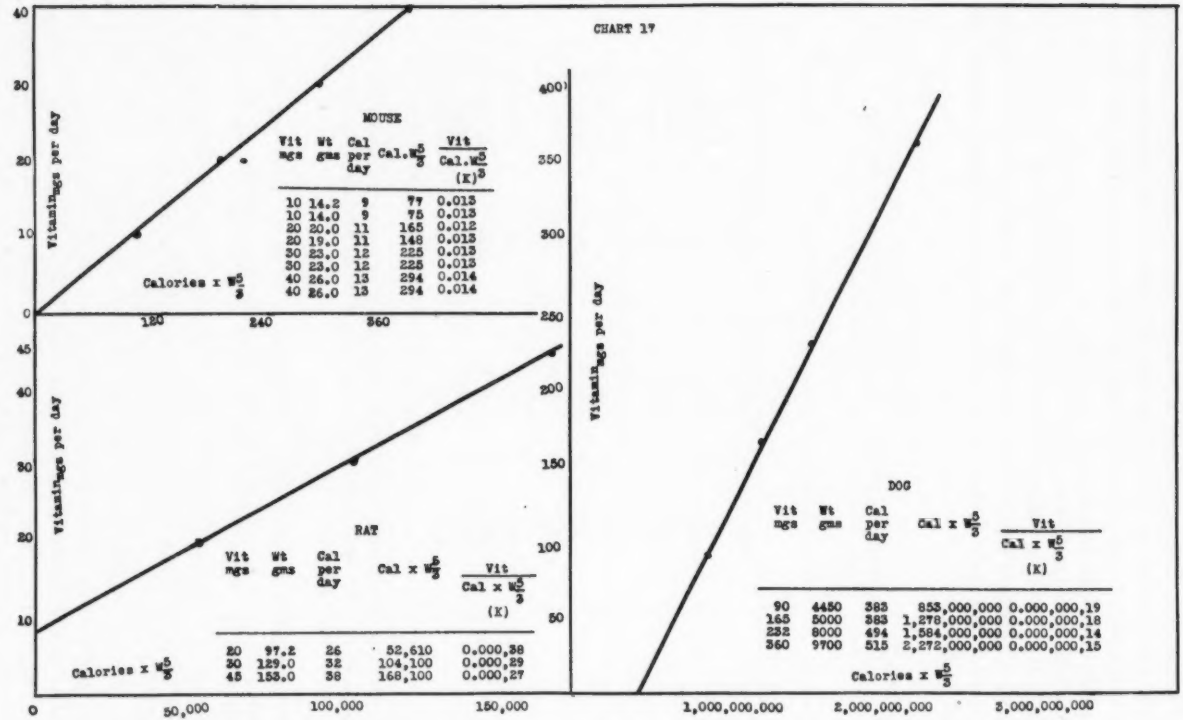

Chart 17. The relation between vitamin (mgm. per day) and calories × weight $\frac{5}{3}$, in the mouse, rat and dog.

in weight, but with a rapid increase when the third was added. When factor C is added to the diet containing A and B, the resumption of growth is much quicker than it is by adding A or B. As sources of vitamins he used cod liver oil, oats and lemon juice.

EXPERIMENTAL. The following diet, free from vitamin C, was fed to three mice for a period of 90 days:

	grams
Soy bean gruel flour and maysoya* (mixed in equal parts)†	86
Cod liver oil	5
Dried yeast	3
Calcium lactate	3
Sodium chloride	3

* The maysoya is composed of undegerminated corn, and soy beans from which the hulls have been removed. Analysis of this product reported by the Cereo Co., Tappan, N. Y., is as follows:

	per cent
Protein	17
Fat	9
Carbohydrate	60
Ash	5
($CaCO_3$; $Ca_3(PO_4)_2$; CaF_2)	
Iron, present.	

† The mixture of soy bean gruel flour and maysoya was autoclaved for 30 minutes at 15 pounds pressure. The other ingredients were then added and mixed, a little water being used to make the mass pack together. Small cakes were then made and dried, and the resulting "soy bean cracker" fed to the animals. (See B. Cohen and L. B. Mendel. Journ. Biol. Chem., 1918, xxxv, 425.)

Chart 18 shows the growth data of all the vitamin C experiments. On the above diet, which was fed simultaneously to guinea pigs weighing 150 grams and which caused their death from scurvy in three weeks, the mice grew slowly but in no case was there found any pathological evidence of scurvy.

Three mice were then placed on the "standard" diet and 15 cc. of fresh orange juice were given daily instead of water for a period of 90 days after weaning. The control animals received the "standard" diet only. Both the juice and pulp in most cases were consumed by the experimental animals, and in two cases growth was subnormal. This was apparently due to the fact that owing to the bulk of the orange juice, these animals did not eat enough of the standard diet to permit normal growth. To test out this point the next three mice were given the same diet as those above but with only 5 cc. of fresh orange juice daily. Tap water was also given in a separate cup. These animals made better growth than those receiving the larger quantity of orange juice above, and in the majority of cases growth was no better than that secured with the "standard" diet alone.

Discussion. The animals in Tönnis investigation received 0.5 cc. of lemon juice daily in their drinking water, while those in the present in-

vestigation should have received enough vitamin C in the fresh orange juice to show the effect of adding this vitamin to the "standard" diet. As fruit juices also contain vitamins A and B, it is easily possible, if Groebbles and Tönnis used too small amounts of these factors in their control diets, that the lemon juice might have caused improved growth by supplying added vitamin A or B or both, rather than vitamin C.

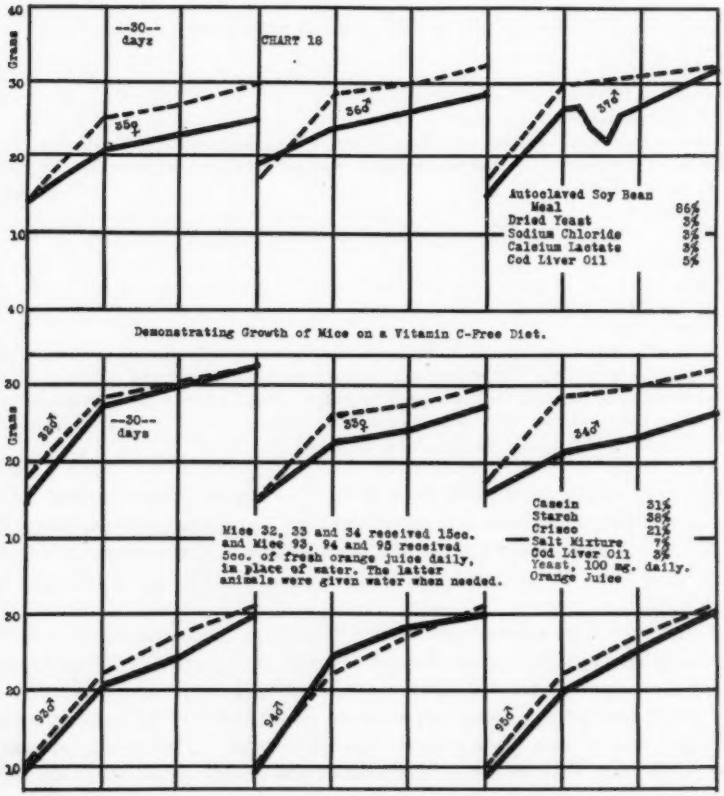

Chart 18. Demonstrating growth of mice on the "standard" diet with the daily addition of fresh orange juice.

Conclusions. By comparing the two series of experiments it seems probable that vitamin C is *not* essential to the normal growth of the animals used in this investigation. This result does not bear out the conclusion of Groebbles and Tönnis that all three vitamins A, B and C are required for the normal growth of the mouse.

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STUDIES IN THE NUTRITION OF THE WHITE MOUSE[1]

IV. The Relation Between Diet and Reproduction

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It has long been known that fertility and successful reproduction of animals depend, among other things, upon the diet. Most of the earlier researches, however, laid emphasis either upon the growth or reproduction resulting from a diet, or upon the internal secretions of the ovaries, testes and thymus, in this connection.

Appreciation of the true relation between diet and reproduction is due to Evans and Bishop (1922) who reported body weight changes of the mother and litter on various complete diets, and found that the classic basic ration failed to give normal fertility in female rats. The defect centered itself upon the implantations of which about 80 per cent may be resorbed. The pathology of the sterility involves "a highly peculiar deficiency disease of the placenta cured by the administration of certain foods." Some of these foods were lettuce, meat, wheat germ, rolled oats, alfalfa and large amounts of milk fat, while whole milk, cod liver oil, orange juice and yeast were not curative, and no evidence was found that the salt content of the diet or its proportion of protein, fat or carbohydrate was at fault. These investigators stated that the unknown factor was distinct from vitamins A, B, C and D, was resistant to cooking and was not stored in quantity by the animal.

When the animals were changed from an adequate diet to a "pure" one deprived of X, they maintained their fertility for a time and then lost it, and when sterile animals were cured by the adequate diet, the fertility imparted to them survived for two generations. Evans and Bishop also reported (1924) studies on the chemical properties of this new vitamin and found that it was soluble in alcohol, ether and acetone, and was therefore called fat-soluble vitamin E.

That sufficient amounts of vitamins A and B are needed for normal reproduction of animals has been shown by several investigators (Evans and

[1] The experimental data in this paper are taken from the dissertation submitted by Howard H. Beard in partial fulfilment of the requirement for the Degree of Doctor of Philosophy, Yale University, 1925.

[2] Porter Fellow of the American Physiological Society, 1924–25.

Bishop, 1923; Kennedy and Palmer, 1922; Sherman and MacLeod, 1924). There is also much evidence, however, that there is another factor, in addition to the above vitamins, necessary in this connection. Mattill and Carman (1923), for instance, found that the degeneration of the testes in rats on a milk diet could not be due to a lack of vitamin B, since it occurred when the liver and kidney were added. They stated that some

TABLE 7

FIRST SERIES

Reproduction of female mice on the stock diet

(Skim milk powder 50; wheat bran 50; dog biscuit and lettuce three times a week; fresh milk daily during lactation)

(First period, 56 days, mated with stock males)

MOUSE NUMBER ♀	AGE AT MATING	NUMBER OF YOUNG		WEIGHT OF YOUNG		WEIGHT OF MOTHER		REMARKS
		At birth	At weaning	At birth	At weaning	After littering	At weaning	
	days			*grams*	*grams*	*grams*	*grams*	
325	21	8	8	14.0	62.0	38.0	32.0	
326	21	10	8	14.0	63.0	31.0	25.0	1 killed, 1 eaten by mother
327	21	8	6	11.0	58.0	34.0	33.0	2 eaten by mother
328	21	6	5	8.5	46.0	29.0	32.0	1 eaten by mother
329	21	8	6	12.0	56.0	29.0	32.0	2 eaten by mother
330	21	8	7	12.0	57.5	28.0	29.0	1 eaten by mother
331	18	9	9	13.0	86.0	31.5	28.0	
333	18	12	9	17.0	86.5	36.0	38.0	3 eaten by mother
334	18	8	7	10.0	67.3	30.0	32.0	1 born dead
335	18	7	4	11.6	36.5	36.0	40.5	1 eaten by mother, 2 died 2nd day
336	18	7	7	11.0	63.5	29.0	32.0	
337	18	8	3	13.0	27.0	29.0	32.0	3 died, 2 eaten by mother
338	21	10	10	14.5	70.0	30.0	35.0	
339	21	7	6	11.4	56.5	28.0	31.0	1 killed by mother
340	21	8	8	10.5	76.3	32.0	34.0	
341	21	9	7	11.7	69.5	36.0	32.0	2 eaten by mother
342	21	9	6	14.0	52.0	32.0	36.0	1 died, 2 eaten by mother
343	21	6	6	9.0	60.0	32.0	31.0	
344	21	7	7	11.0	67.5	36.0	33.0	

other unknown substance was responsible for the disappearance of the reproductive function. In a later paper Mattill and Congdon (1924) found that the improved reproductive function of female rats, resulting from the addition of yeast to milk rations, was due neither to yeast nucleic acid nor vitamin B.

Mattill, Carman and Clayton (1924) stated that sterility on milk rations affects both sexes. In the female implantation was unsuccessful while

ovulation was normal. In the male there was a progressive degeneration of the testes observed after 100 days. Reproductive failure did not occur when the diet was supplemented with 5 to 10 per cent wheat embryo or lettuce. These investigators stated that the curative principle resided in the fat of the wheat embryo, and considered it the "X" substance of Evans.

TABLE 7 (a)

FIRST SERIES

Reproduction of female mice on the stock diet

(Skim milk powder 50; wheat bran 50; dog biscuit and lettuce three times a week; fresh milk during lactation)

(Second period, 60 days, mated with experimental males on the "standard" diet)

STOCK MOUSE NUMBER ♀	EXPERIMENTAL MOUSE NUMBER ♂	NUMBER OF YOUNG		WEIGHT OF YOUNG		WEIGHT OF MOTHER		REMARKS
		At birth	At weaning	At birth	At weaning	After littering	At weaning	
				grams	*grams*	*grams*	*grams*	
325	214	4	4	7.0	30.0	34.0	32.0	
326	158							No young second period
327	158	9	0	12.0		27.0	29.0	3 died at birth, 6 eaten by mother
328	137							No young second period
329	206	7	7	11.0	64.0	27.0	34.0	
330	137							No young second period
331	202							No young second period
333	207							No young second period
334	212							No young second period
335	214	4	2	7.0	16.0	35.0	36.0	1 dead, 1 eaten by mother
		6	6	8.0	34.0	34.0	35.0	
336	173							No young second period
337	216	9	0	13.0		27.0	31.0	5 died, 4 eaten by mother
338	205							No young second period
339	202							No young second period
340	210							No young second period
341	214	10	10	14.0	68.0	36.0	31.0	
342	202							No young second period
343	212							No young second period
344	212							No young second period

The degeneration of the testes of rats raised on the "standard" casein diet has been reported by Mason (1925), who found that it appeared after a period of 60 to 100 days. The sperm and spermatids are first affected, and in later stages the entire germinal epithelium, while the Sertoli cells remained normal.

EXPERIMENTAL. The mice used in the present investigations were fed the same "standard" diet as that used by Mason, except that a concentra-

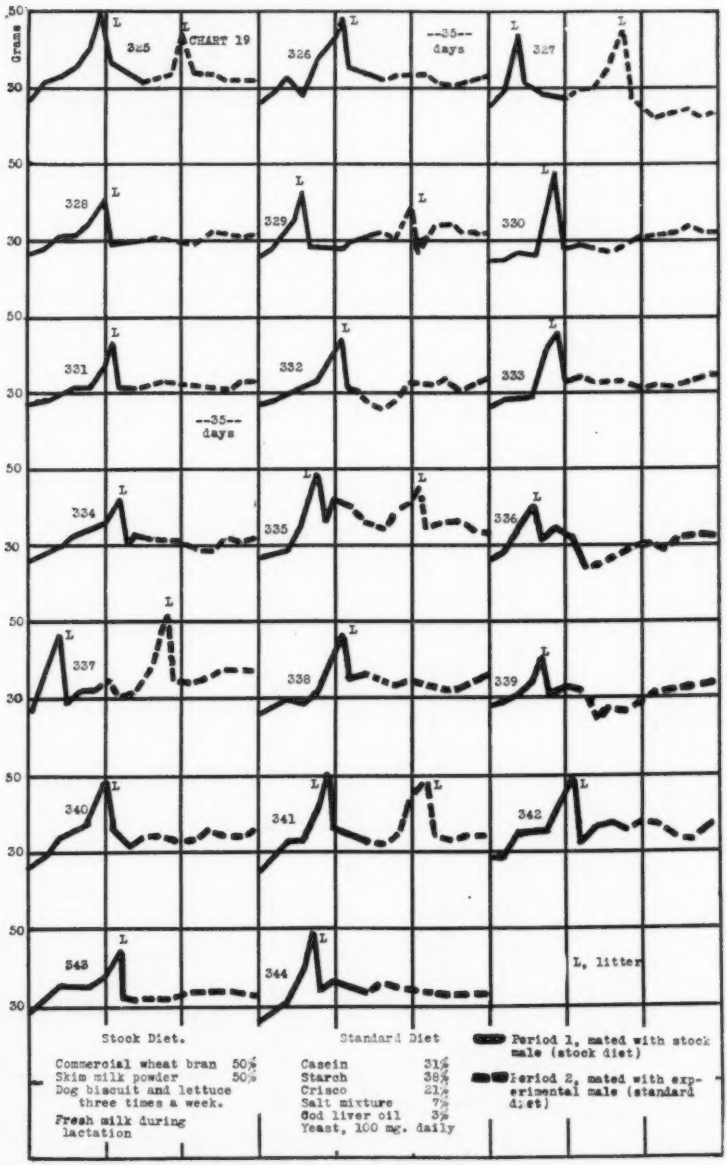

Chart 19. Reproduction of stock females: 1, with males on the "stock" diet; 2, with experimental males on the "standard" diet.

TABLE 8

SECOND SERIES

Reproduction of female mice on the "standard" diet

(Casein 31, starch 38, crisco 21, salt mixture 7, cod liver oil 3, dried yeast 100 mgm. tablet daily)

FIRST PERIOD, 49 DAYS MATED WITH EXPERIMENTAL MALES, ON "STANDARD" DIET								SECOND PERIOD, 56 DAYS MATED WITH STOCK MALES, ON "STOCK" DIET							
Mouse number ♀	Number of young		Weight of young		Weight of mother		Remarks	Stock mouse number ♂	Number of young		Weight of young		Weight of mother		Remarks
	At birth	At weaning	At birth	At weaning	After littering	At weaning			At birth	At weaning	At birth	At weaning	After littering	At weaning	
			grams	*grams*	*grams*	*grams*					*grams*	*grams*	*grams*	*grams*	
137								325							
158								327							
165								327							
204	8	0	11.0	0	29.0	27.0	4 born dead, 4 eaten by mother	333	2	0	3.0	0			2 born dead
202								331	4	0	5.0	0			All dead at birth
173	6	0	11.0				All eaten by mother	329							
205								335							
206								337	6	0	7.5	0			2 dead, 4 eaten by mother
207								335	6	0	7.0	0			2 born dead, 4 died later
208								339							
210								338	6	0	9.0	0			3 eaten by mother, 3 died
214								341	6	0	8.5	0			3 dead, 3 eaten by mother

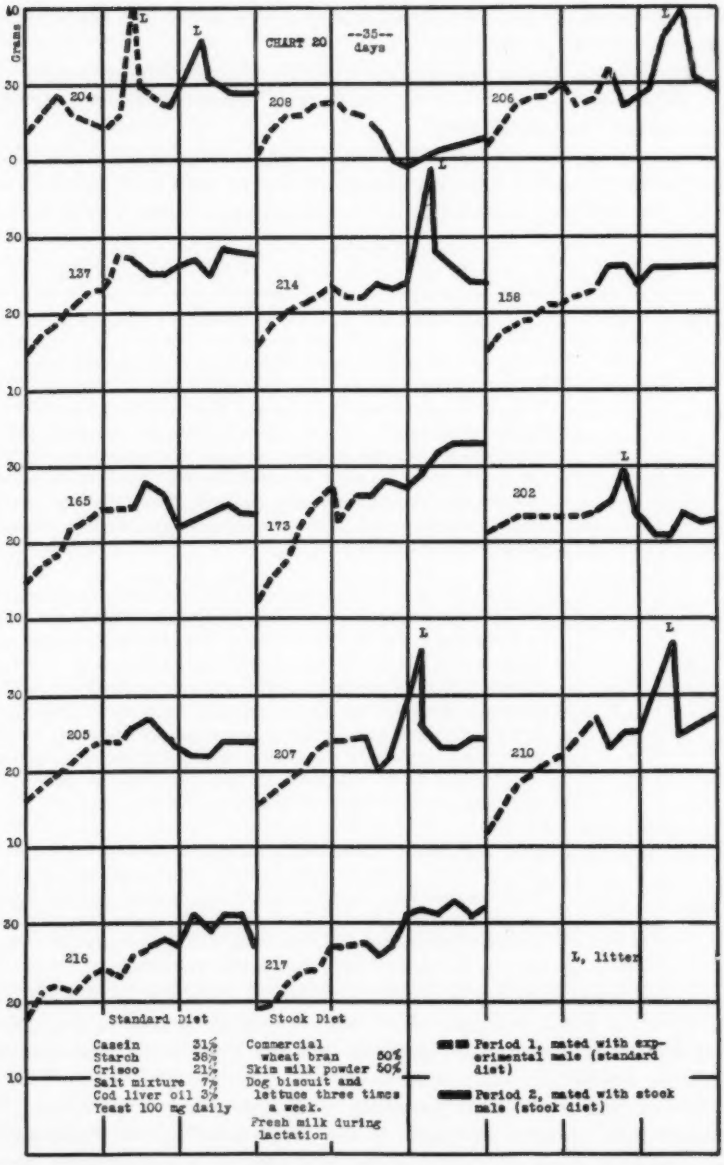

Chart 20. Reproduction of experimental females: 1, with males on the "standard" diet; 2, with males on the "stock" diet.

tion of 31 per cent casein was used, and in this instance was not purified by extraction with alcohol and ether.

In the experiments reported below "stock" males and females refer to mice fed the stock diet, while "experimental" males and females signify those fed the "standard" diet.

Series 1, group 1. Twenty-nine "stock" mice were mated in the first period on the stock diet, consisting of equal parts of skim milk powder and wheat bran, with dog biscuit and lettuce added three times a week (chart

TABLE 9

The histo-pathology of the testes of mice raised on the "standard" diet

MOUSE NUMBER	DAYS ON DIET	TESTES
223	105	Practically normal. A few tubules show slight degeneration.
219	105	Slightly degenerate. Practically every tubule appears normal, but on closer observation, many show a reduced number of sperm (due to the slowing down of the spermatogenic process), and many such sperm show slight degenerative changes. In a few other tubules occasionally a few spermatids and spermatocytes are abnormal, but this may sometimes occur in normal animals. Germinal epithelium slightly vacuolated in a few tubules.
205	180	About 50 per cent of tubules appear fairly normal, but in most cases the spermatozoa are undergoing degenerative changes. A large number of the other tubules have lost their sperm and spermatids, except for the degenerated remains of a few of these, and only spermatogonia and spermatocytes (chiefly primary) are left. A few tubules also show occasional giant cells of different sizes. Degenerated tubules are not so markedly vacuolated as in the case of mouse 214 below. None of the tubules have reached complete degeneration (stage 5), and only a few show as advanced a condition as stage 4.
214	180	Badly degenerate. Practically no sperm present except in a few tubules. Some tubules contain other germ cells in normal numbers and appearance, but the majority of which are extremely vacuolated and contain chiefly spermatogonia and primary spermatocytes, which do not appear normal. Very similar to stage 4, but giant cells rare and small.

19, table 7). Fresh milk was also added during the period of lactation. A total of 19 litters was born showing that the 29 animals used were all of proven fertility.

Series 2, group 2. Simultaneously with the above experiment, 21 "experimental" mice were mated in the first period on the "standard" diet (chart 20, table 8). Two litters containing 14 young were born, none of which were weaned.

The object of these studies was to determine the extent of the sterility in both sexes. In the second period, therefore, all of the above animals, 50 in number, were placed in separate cages; the "stock" males were then placed each night in the cages of the "experimental" females and next morning returned to their own cages. In like manner the "stock" females were placed in the cages of the "experimental" males.

In the case of the experimental males and stock females mated in the second period (chart 19, table 7a), there were 7 litters containing 49 young of which 59.2 per cent were successfully weaned, while in the case of the experimental females mated with the stock males, no young were weaned. These results would indicate that in the female the standard diet causes sterility similar to that observed by Evans in rats.

The testes of a number of the "experimental" males used above were prepared for histological study, as follows:

Both testes, one of which was practically cut in two, were placed in Bouin's mixture for 14 to 16 hours, the mixture then poured off, and the tissues cleared by means of 70 per cent alcohol with the aid of a few drops of lithium carbonate, then dehydrated through 95 and 100 per cent alcohol, cleared in xylol, passed through three changes of paraffin, and then embedded in hard paraffin with a M.P. of 56 to 58°C.

Sections were cut 5 to 7 micra in thickness, mounted, placed in 4 per cent iron alum for 6 hours, washed, and stained with Heidenhain's iron hematoxylin for 10 to 15 hours. After removing the excess stain in distilled water the sections were again placed in the iron alum until the proper amount of stain remained. They were then placed in alcohol of increasing strengths, and finally in xylol, and mounted in balsam for study.

Table 9 shows the results of some of the histological examinations, from which it is evident that the degeneration of the testes in mice is similar to that observed by Mason in the testes of rats (except that it appears later in the former than it does in the latter), when both species are fed upon the "standard" diet under similar conditions. This may be due to the fact that the rats mentioned above were fed upon purified casein; however, further work must be done in this connection to obtain the complete histological picture in the testes of mice when fed upon the "standard" diet.

Series 3. In the first period the animals were fed the "standard" diet for a period of 56 days (chart 21, table 10). Only 5 young were born, none of which were weaned. Lettuce was then added three times a week in the second period for 147 days, during which time 117 young were born, 55 per cent of which were successfully weaned, thus showing a marked improvement in reproduction. The average weight of the young at weaning was 7.5 grams as compared with 9 grams for the normal, and the mothers suffered an average loss of 3.7 grams in body weight during lactation. This indicates that the "standard" diet, with the addition of lettuce, is not sufficient for optimum lactation in mice.

TABLE 10

THIRD SERIES

Reproduction of female mice on the "standard" diet, and after sterility was proven, fed in addition fresh lettuce three times a week

FIRST PERIOD, 56 DAYS		SECOND PERIOD, 147 DAYS						
		Gestation on "standard" diet plus lettuce						
		Number of young		Weight of young		Weight of mother		
Mouse number	Gestations on "standard" diet	At birth	At weaning	At birth	At weaning	After littering	At weaning	Remarks
				grams	*grams*	*grams*	*grams*	
219		4	0	6.0	0.0	28.0	26.0	All died 2nd day
220		9	4	11.5	26.0	31.0	28.0	5 died first week
		7	5	10.0	30.0	32.0	32.0	2 eaten by mother
221		2	0	3.0	0 0	30.0	27.0	All died 3rd day
		9	8	12.0	53.0	33.0	29.0	1 died
222	1 born dead	4	0	5.0	0.0	30.0	31.0	All died 2nd day
		1	0	1.2	0.0	30.0	30.0	Died 1st day
223		6	0	6.0	0.0	27.0	26.0	2 born dead, 2 died later
224								No young
225		6	5	10.0	47.0	30.0	25.0	1 dead at birth
		6	6	9.0	54.0	30.0	26.0	
226	4, 2 dead, 2 eaten by mother	6	0	8.0	0.0	32.0	34.0	All dead 2nd day
		8	6	12.5	40.0	36.0	26.0	2 eaten by mother
227		3	0	4.0	0.0	38.0	25.0	All died 3rd day
		8	7	8.5	42.0	27.0	25.0	1 eaten by mother
228		7	2	11.0	15.0	30.5	27.0	5 eaten by mother
		9	5	10.5	46.0	34.0	34.0	2 dead at birth, 2 died
233		6	4	8.0	36.0	31.0	32.0	2 dead at birth
234		2	0	3.0	0.0	33.0	30.0	All eaten by mother
		6	6	7.5	53.5	30.0	32.0	
235		8	6	12.0	42.0	33.0	28.0	2 dead at birth
236								No young

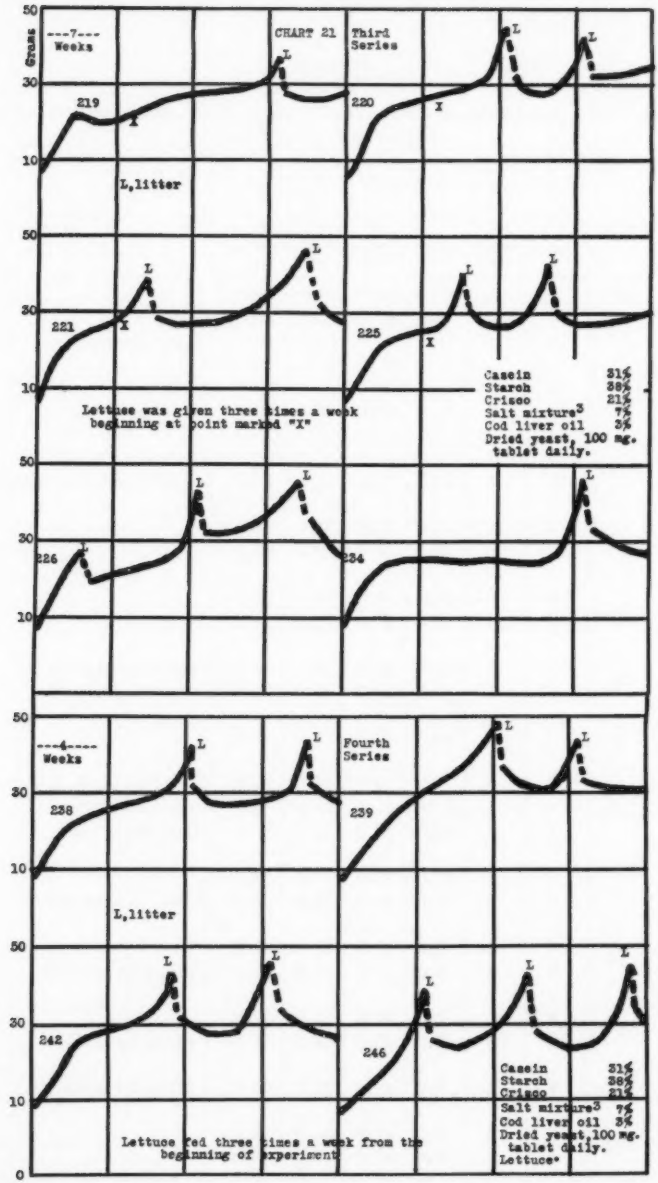

Chart 21. Reproduction of experimental females on the "standard" diet, with the addition of lettuce.

TABLE 11

FOURTH SERIES

Reproduction of female mice on the "standard" diet with the addition of lettuce from the beginning of experiment

MOUSE NUMBER	AGE AT MATING	NUMBER OF YOUNG		WEIGHT OF YOUNG		WEIGHT OF MOTHER		REMARKS
		At birth	At weaning	At birth	At weaning	After littering	At weaning	
	days			*grams*	*grams*	*grams*	*grams*	
238	18	6	6	8.5	40.0	33.0	27.0	
		8	6	11.0	44.0	31.0	28.0	2 dead at birth
239	18	8	0	12.5	0.0	36.0	30.0	All killed by mother
		8	6	11.5	48.0	32.0	30.0	2 dead at birth
240	18	5	0	7.0	0.0	32.0	32.0	All dead at birth
		10	7	12.0	50.0	33.0	27.0	1 died, 2 eaten by mother
241	18	5	5	5.0	38.0	31.0	28.0	
		7	7	9.5	54.0	33.0	30.0	
242	18	8	8	12.0	56.0	30.0	27.0	
		6	6	8.5	47.0	33.0	28.0	
243	18	8	7	11.0	42.0	34.0	30.0	1 eaten by mother
		6	6	9.3	30.0	30.0	27.0	Litter 14 days old
245	18	7	7	10.0	52.0	28.0	23.0	
246	18	7	0	9.0	0.0	27.0	25.0	3 dead at birth, 4 died later
		8	5	11.5	37.5	28.0	24.0	3 dead at birth
		7	7	10.0	18.0	32.0	27.0	Litter 6 days old
247	18	7	7	11.0	63.0	31.0	28.0	
		8	8	11.0	42.0	36.0	34.0	Litter 22 days old
248	18	6	6	8.0	36.0	37.0	34.0	
		5	3	7.0	24.0	35.0	34.0	2 killed by mother
249	20	7	7	9.0	42.0	34.0	32.0	
		6	5	7.0	35.0	36.0	31.0	1 eaten by mother
250	20	7	5	9.0	40.5	37.0	35.0	2 killed by mother
251	20	6	6	9.0	48.0	36 0	35.0	
		6	6	8.0	43.0	32.0	30.0	

TABLE 11—*Concluded*

MOUSE NUMBER	AGE AT MATING	NUMBER OF YOUNG		WEIGHT OF YOUNG		WEIGHT OF MOTHER		REMARKS
		At birth	At weaning	At birth	At weaning	After littering	At weaning	
	days			*grams*	*grams*	*grams*	*grams*	
252	20	8	7	11.0	56.0	37.0	33.0	1 died 2nd day
253	20	7	6	9.0	48.0	36.0	34.0	1 born dead
		6	6	8.0	43.0	35.0	32.0	
254	17	8	4	11.0	29.0	32.0	30.0	2 born dead, 2 eaten by mother
		4	4	6.0	31.0	35 0	35 0	
255	17	7	6	9.0	42.0	34.0	30.0	1 killed by mother
		5	5	8.0	40.0	36.0	32.0	
256	17	6	6	8.0	36.0	37.0	34.0	
257	17	6	6	7.5	41.0	35.0	33.0	
		7	7	9.0	56.0	35.0	34.0	
258	17	3	0	5.0	0.0	34.0	32.0	All died 3rd day

TABLE 12

Containing a summary of tables 7, 7a, 8, 10 and 11

	NUMBER OF ANIMALS USED	TOTAL NUMBER YOUNG		PER CENT WEANED	AVERAGE WEIGHT OF YOUNG		AVERAGE GAIN OR LOSS OF MOTHER'S WEIGHT DURING LACTATION	NUMBER KILLED BY MOTHER	NUMBER EATEN BY MOTHER	NUMBER BORN DEAD	NUMBER DYING AFTER BIRTH
		Born	Weaned		At birth	At weaning					
					grams	*grams*					
Table 7, first period	19	155	129	83.2	1.5	9.0		2	17	1	6
Table 7 (a), second period	19	49	29	59.2	1.4	7.3	0.2	0	11	1	8
Table 8:											
First period	14	14	0	0.0	1.4	0.0		0	8	4	2
Second period	14	30	0	0.0	1.3	0.0		0	10	13	7
Table 10:											
First period	14	5	0	0.0	0.0	0.0		0	2	3	0
Second period	14	117	64	55.0	1.4	7.5	−3.7	0	12	9	32
Table 11	20	239	193	81.0	1.3	6.2	−5.2	13	6	18	9

Series 4. The animals of these experiments were given the "standard" diet with the addition of lettuce three times a week, from the beginning (chart 21, table 11). Table 12 (which contains a summary of all the studies reported in this paper) shows that a total of 239 young were born, 81 per cent of which were successfully weaned, which compares favorably with 83.2 per cent from animals on the "stock" diet (table 7, first period). The average weight at weaning was 6.2 grams, and the average loss in the mother's weight during lactation was 5.2 grams, which again shows that the "standard" diet is insufficient for the nutritive needs of mice during this period.

Two litters were obtained in most cases from both series 3 and 4 reported above. Further studies in this connection will be reported later.

Series 5. The animals in this series were fed fresh milk only. Growth was subnormal and no young were born. The addition of the four inorganic salts in the same proportion as used by Daniels and Hutton (1925) did not induce any reproduction. Another group was fed upon whole milk powder as the sole diet, with the same results.

CONCLUSIONS

Definite evidence has been obtained from the above investigations that there is another factor, in addition to the proteins, fats, carbohydrates, salts, vitamins A and B, necessary for normal reproduction in mice. The sterility was shown to be effective in both sexes, possibly greater in the female than in the male. These results confirm the view of Evans and Bishop that there is a new factor, possibly a vitamin E (or X), necessary for normal reproduction when rats are fed upon a purified diet.

Histological examination of the testes of the males in some of the above studies revealed a slight degeneration after 105 days on the "standard" diet, and a more marked degeneration after 180 days, probably resembling stage 4 of Mason's studies on the testes of rats.

Fresh green lettuce fed three times a week with the "standard" diet was effective in curing sterility, and also in promoting normal reproduction in mice, but did not induce adequate normal lactation.

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I wish to express my thanks to Prof. Lafayette B. Mendel for suggesting the general problem and furnishing advice regarding many details. Professors Arthur H. Smith and George R. Cowgill have assisted me generously in the conduct of the work.

Grateful acknowledgment is also due Dr. E. A. Park and Miss Deborah Jackson, of the Department of Pediatrics, who made the histological

examination of the bones of animals fed upon rachitic diets; to Dr. A. M. Yudkin, of the Department of Ophthamology, for the examination of the eyes of some of the mice fed on vitamin-A-free diets, and to Dr. Karl E. Mason, of the Osborne Zoölogical Laboratory, under whose direction the histological work upon the testes in the reproduction studies was made.

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MINERAL METABOLISM STUDIES WITH DAIRY CATTLE[1]

Mineral Equilibrium During Gestation

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In our studies on mineral balance during early (Miller, 1924) and late (Miller, 1925) lactation we were able to compare the metabolism of minerals of the three cows at approximately the same time in their lactation period. This condition during the gestation period could not be realized and considering the circumstances we were obliged to study the mineral balance of animal 216 fifteen weeks before parturition. Animal 222 calved seven days after one ten-day period observation of the mineral balance status on the basal ration and animal 218 was in the thirteenth day of lactation. While our data obtained relating to the storage of minerals during gestation and no milk yield are not so extensive as those for the particular time in the other periods, they have given the opportunity to observe absorption of minerals from the alimentary tract and their retention when the demands of the animal would be expected to vary, due to difference of a period or more advanced stage in a particular period. Animal 218 being in early lactation again gave the opportunity to confirm what we had observed during the former lactation period.

Experimental. Except for a few weeks when corn silage was substituted for the oats and vetch silage the feeds selected for the ration during the intervening periods were the same as when the intake and excretion of minerals were under quantitative observation. The mineral balance period reported here was of 27 days duration. Three days' preliminary feeding on the definite quantities of ration were followed by a ten-day period during which an accurate record of the total intake and outgo of minerals on the basal ration was kept. The ration was then supplemented with bone meal and after 4 days, the quantitative observation was continued over another ten-day period.

Animal 218 which was producing about 23.0 kilograms of milk daily was given 12.0 pounds of red clover hay, 13 pounds of grain, (corn, 58, millrun 32, oil meal 10), 30.0 pounds of oats and vetch silage and 20.0 pounds of

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green kale daily. Common salt was added in the proportion of 1.25 pounds of salt to 100 pounds of grain. Differing from the first observation during early lactation of 218, kale was given throughout the time of this experiment and during the second ten-day period the ration was supplemented with 150 grams of bone meal daily. Animals 216 and 222 which

TABLE 1

Average daily mineral balance recorded in grams

PERIOD	NITROGEN	SULFUR	PHOSPHORUS	CHLORINE	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM
Animal 216:								
Gestation, dry, basal ration	202.8*	19.6	35.6	34.8	98.8	34.4	181.1	23.9
	106.1	4.0	0.5	19.5	0.4	6.8	168.8	7.5
	76.0	8.6	31.1	13.2	83.8	24.8	8.4	3.1
	+20.7	+7.0	+4.0	+2.1	+14.6	+2.8	+3.9	+13.3
Gestation, dry, basal ration plus bone meal	204.6	19.6	58.9	35.3	142.3	35.2	181.1	24.2
	107.4	4.5	0.8	22.0	0.6	6.6	170.0	12.5
	77.6	9.6	53.0	14.4	136.0	27.9	8.3	3.4
	+19.6	+5.5	+5.1	−1.1	+5.7	+0.7	+2.8	+8.3
Animal 222:								
Gestation, dry, basal ration	198.4	19.2	34.7	34.0	95.9	33.7	173.6	23.4
	86.4	3.8	0.9	13.8	0.5	7.8	153.6	5.7
	71.6	8.5	25.4	12.8	75.6	23.4	11.4	2.6
	+40.4	+6.9	+8.4	+7.4	+19.8	+2.5	+8.6	+15.1
Animal 218:								
Early lactation, 23.15 kilos milk daily, basal plus kale	315.6	33.5	58.5	72.9	117.0	45.0	226.4	43.1
	109.7†	6.4	22.3	17.7	27.3	2.8	28.0	8.1
	115.7	9.7	0.7	38.0	0.8	8.5	178.8	16.7
	108.7	12.0	36.7	18.3	95.1	36.0	17.1	4.9
	−18.5	+5.4	−1.2	−1.1	−6.2	−2.3	+2.5	+13.4
Early lactation, 23.58 kilos milk daily, basal, plus kale, bone meal	316.8	33.5	81.8	72.5	160.5	45.6	223.4	43.3
	102.8	6.4	20.7	20.0	26.0	2.8	26.0	8.3
	111.7	8.0	0.5	37.2	0.3	8.4	184.0	18.9
	108.2	13.0	59.6	18.1	130.5	34.5	19.1	4.6
	−5.9	+6.1	+1.0	−2.8	+3.7	−0.1	−5.7	+11.5

* Quantities in feed, urine, feces, balance
† Milk.

were dry received 12.0 pounds of hay, 30 pounds of silage and 6.0 pounds of the grain mixture containing common salt in the proportion of one pound of salt to 100 pounds grain. During the second ten-day period animal 216 received 150 grams of bone meal daily. Because of the approach of parturition time it was necessary to discontinue the mineral balance observation with animal 222.

TABLE 2

Daily intake and absorption of certain elements

PERIOD	ANIMAL	RATION	DAILY MILK YIELDS	CALCIUM			PHOSPHORUS			MAGNESIUM			NITROGEN			SULFUR		
				Grams in feed	Grams absorbed from intestine	Percentage absorption	Grams in feed	Grams absorbed from intestine	Percentage absorption	Grams in feed	Grams absorbed from intestine	Percentage absorption	Grams in feed	Grams absorbed from intestine	Percentage absorbed	Grams in feed	Grams absorbed from intestine	Percentage absorbed
			kilos															
Early lactation	216	Basal*	19.55	72.9	16.9	23.2	47.7	16.7	35.0	32.1	8.0	25.0	208.4	117.6	56.4	17.0	8.5	50.0
	222	Basal	18.93	71.9	16.9	23.5	46.6	15.5	33.2	31.6	10.0	31.6	204.1	120.1	58.9	16.6	9.0	54.2
	218	Basal	20.64	73.3	15.8	21.6	52.9	14.9	28.2	34.4	9.7	28.2	225.0	125.1	55.6	18.3	8.9	48.6
	216	Basal, bone meal	19.04	117.5	24.5	20.8	74.6	25.3	33.9	34.4	8.4	24.4	219.0	119.7	54.6	18.2	9.2	50.5
	222	Basal, bone meal	19.83	118.3	27.7	23.4	71.5	25.7	35.9	33.2	9.2	27.7	209.6	119.4	57.1	17.4	8.7	50.0
	218	Basal, kale	23.29	94.1	20.2	21.4	60.4	23.3	38.6	39.0	10.7	27.4	275.3	163.9	59.5	26.9	16.1	59.8
Prolonged lactation	216	Basal	9.25	90.3	11.2	12.4	34.0	11.9	35.0	30.0	3.2	10.6	214.8	122.1	56.7	16.1	6.7	41.5
	222	Basal	9.84	90.5	11.4	12.6	35.9	11.9	33.2	34.1	8.6	25.2	222.8	134.8	60.0	16.8	7.7	45.8
	218	Basal	10.40	90.6	18.6	9.5	36.6	12.2	33.3	34.4	7.7	22.4	225.7	125.8	55.8	17.0	8.2	48.2
	216	Basal, bone meal	9.00	132.3	24.3	18.4	57.1	16.5	29.0	33.9	4.6	13.6	216.0	113.9	52.7	16.6	6.7	40.4
	222	Basal, bone meal	9.05	131.6	19.5	14.8	58.8	14.3	24.4	34.5	9.4	27.2	220.0	134.3	61.1	17.1	7.5	44.0
	218	Basal, kale	11.92	107.1	20.3	18.9	40.0	11.7	29.2	37.1	9.9	26.7	251.8	153.1	60.1	22.9	11.9	52.0
Gestation	216	Basal		98.8	15.0	15.4	35.6	4.5	12.6	34.4	9.6	27.9	202.8	126.8	62.5	19.6	11.0	56.2
	222	Basal		95.9	20.3	21.2	34.7	9.3	26.8	33.7	10.3	30.6	198.4	126.8	64.0	19.2	10.7	55.6
	216	Basal, bone meal		142.3	6.3	4.4	58.9	5.9	10.0	35.2	7.3	20.7	204.6	127.0	62.0	19.6	10.0	51.0
Lactation	218	Basal, kale	23.15	117.0	21.9	18.7	58.5	21.8	37.3	45.0	9.0	20.0	215.6	206.9	65.6	33.5	21.5	64.2
	218	Basal, kale, bone meal	23.58	160.5	30.0	18.7	81.8	22.2	27.1	45.6	11.1	24.4	316.8	208.6	66.0	33.5	20.5	61.1

* Red clover, oats and vetch silage, grain.

DISCUSSION OF RESULTS. With the elements calcium, phosphorus and chlorine with which we are chiefly concerned in this mineral metabolism work, the condition of equilibrium as shown in table 1 is approximately the same during the early lactation period of animal 218 while being fed the basal ration plus kale as in a similar period formerly reported. The high proportion of phosphorus absorbed and the favorable balance of this element are revealed in both cases. The calcium and chlorine though fed on a higher plane this time are again negative. Supplementing the ration with bone meal brought about positive calcium and phosphorus balances with this animal similar to what was observed with animals 216 and 222 during lactation. Data in table 1 show the marked storage of all minerals on the basal ration during the gestation period of animals 216 and 222. The large quantities of calcium and phosphorus retained by animal 222, a few days prior to parturition, are especially noticeable. Animal 216 also stored liberal quantities of the inorganic constituents from the basal ration fifteen weeks before parturition. The decrease in calcium retention on supplementing her ration with bone meal was surprising in view of what we had observed before with these animals receiving the same quality of ration. This animal being in the middle of gestation period and producing no milk the important fact cannot be overlooked that the demands for the inorganic constituents are not as great as formerly. This is further exemplified by the data in table 2.

Fortunately for comparing the absorption and retention of minerals, the amounts of total elements consumed, the milk production and the age of the lactation period are approximately the same for animals 216 and 222 during the different periods under observation. The calcium and phosphorus not excreted in the feces are used either to augment the body supply or for milk formation. It is interesting to note that the total calcium absorptions of animals 216 and 222 on the basal rations parallel each other during the lactation periods and the quantities absorbed decrease as lactation falls off though the calcium plane of intake was increased. This relationship between calcium absorption and requirements was further demonstrated in the gestation period. Because of the time in the gestation period the requirements of animal 222 for calcium must have been greater than those of animal 216. This assumption is supported by the proportion and total quantity of calcium retained by animal 222 so that a marked difference in the quantities of calcium absorbed resulted whereas in previous periods they paralleled each other for the two animals. The metabolism of phosphorus was apparently controlled by the same factors as the amounts absorbed too changed with the requirements of the animal. The quality of the rations in the above comparisons has not been a limiting factor in comparing the mineral retentions of animals 216 and 222 for each animal received the same nutrients. For the purpose of comparing

the absorption of other elements from the alimentary tract similar data on magnesium, nitrogen and sulphur have been incorporated in table 2. This was simply to demonstrate that there was no noticeable difference of the basal rations used towards intestinal digestion. The availability of calcium and phosphorus apparently did not vary and the retention of these elements was governed by the regulatory mechanism of the intestine, either by absorption alone or absorption and excretion by the same path. There is sufficient evidence to show that changing the quality of the ration or supplementing a ration with other nutrients will modify the metabolism of certain minerals. This was observed in supplementing our rations with kale.

In the early lactation periods kale increased the total and percentage phosphorus absorbed and had a beneficial effect on the phosphorus balance. After prolonged lactation, addition of kale increased the milk yield but did not increase the phosphorus absorption; however, the calcium absorption was markedly increased. This difference was not apparently due to a difference in the properties of the kale but more to the physiological requirements of the animal at this time. Addition of bone meal to the rations of animals 216 and 222 at this time also greatly increased the absorption of calcium and even the percent of calcium absorbed was increased. This latter condition was not realized before although the negative calcium and phosphorus balances were changed to positive by supplementing with bone meal. With all three animals after prolonged lactation the percentage phosphorus absorbed was about the same as in early lactation, 216 and 222 were storing some calcium while 218 was in a negative calcium balance although the plane of calcium intake was higher than in early lactation. While the natural sources of feed used in all the experimental work was the same, the quality of the ration, especially the hay, may have had some influence on calcium absorption in view of the above observations. This would not, however, detract from our evidence pertaining to alimentary absorption of calcium and phosphorus being controlled to a large degree by the requirements of the animal at that particular time. Accompanying the low calcium absorption from the basal ration in the prolonged lactation period, the base and storage result in giving a negative base equilibrium.

The nature of the acid base-equilibrium appears to be governed by the demands of the animal at that particular time and also by the character of the ration. In the early-lactation and gestation periods a positive base equilibrium occurred on the basal ration while for animal 218 during both early-lactation periods there was a negative base equilibrium on the kale ration. The change in the acid-base equilibrium resulting from supplementing with bone meal was the same for all the animals but varied according to the period.

TABLE 3

Average daily mineral balances observed during the different periods

PERIOD	RATION	MILK YIELD	NITROGEN	SULFUR	PHOSPHORUS	CHLORINE	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM
		kilos	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Animal 216:										
Early lactation	Basal*	19.5	−23.5	1.1	−5.0	−1.2	−5.4	1.8	2.4	1.8
Early lactation	Basal, bone meal	19.0	−12.7	2.1	5.4	−1.4	3.8	1.8	1.9	1.0
Prolonged lactation	Basal	9.3	4.2	1.7	2.8	5.2	1.1	−3.2	−5.8	−0.6
Prolonged lactation	Basal, bone meal	9.0	1.7	1.4	7.3	5.7	14.5	−1.5	−6.3	0.6
Gestation	Basal		20.7	7.0	4.0	2.1	14.6	2.8	3.9	13.3
Gestation	Basal, bone meal		19.6	5.5	5.1	−1.1	5.7	0.7	2.8	8.3
Animal 218:										
Early lactation	Basal	20.6	−23.0	1.0	−7.3	0.2	−7.4	2.2	6.0	2.5
Early lactation	Basal, kale	23.3	−1.7	4.0	0.4	−1.4	−4.0	1.9	−0.2	−2.6
Prolonged lactation	Basal	10.4	1.4	2.1	3.0	5.8	−3.2	−1.8	−12.2	−4.8
Prolonged lactation	Basal, kale	11.9	20.3	2.1	1.3	5.8	5.0	0.7	7.8	−0.6
Early lactation	Basal, kale	23.2	−18.5	5.4	−1.2	−1.1	−6.2	−2.3	2.5	13.4
Early lactation	Basal, kale, bone meal	23.6	−5.9	6.1	1.0	−2.8	3.7	−0.1	−5.7	11.5
Animal 222:										
Early lactation	Basal	18.9	−15.1	−1.9	−4.4	3.2	−6.0	3.3	8.3	5.4
Early lactation	Basal, bone meal	19.8	−15.9	1.3	6.5	−3.5	5.8	2.1	−1.9	−1.4
Prolonged lactation	Basal	9.8	11.6	2.5	3.5	5.5	0.4	−0.1	−6.5	−0.4
Prolonged lactation	Basal, bone meal	9.0	19.2	1.9	6.6	3.9	10.0	1.0	−5.0	2.1
Gestation	Basal		40.4	6.9	8.4	7.4	19.8	2.5	8.6	15.1

* Red clover, oats and vetch silage, grain.

A survey of the mineral balances of the different periods as represented in table 3 shows that with the exception of calcium and phosphorus there was no consistency as to the nature of the balances of the minerals resulting from the differences in nature of feed or period in the reproductive cycle. In the early lactation periods the calcium and phosphorus balances were always negative but were changed to positive balances when bone meal was added to the ration. The variation in the plus or negative balances of the other inorganic elements did not follow any specific period in production by the animal or plane of intake, also, the algebraic sum of any of these elements for the total number of periods did not indicate any marked effect on the total body supply through the reproductive cycles. These results together with the storage of calcium and phosphorus with the basal

TABLE 4

Daily base balance expressed in cubic centimeters of N/10 solution per kilogram of weight. 216 and 222 in gestation, 218 early lactation

ANIMAL	RATION	$\frac{N}{10}$ SOLUTION	REMARKS	RATIO ACID:BASE FECES
216	Basal ration	14.3 positive	Acid storage Base storage	1:2.26
	Basal ration plus bone meal	3.0 positive	Acid storage Base storage	1:2.13
218	Basal ration, kale	1.7 negative	Acid storage Base storage	1:2.3
	Basal ration, kale, bone meal	3.6 negative	Acid storage Base storage	1:1.95
222	Basal ration	13.7 positive	Acid storage Base storage	1:2.42

ration during late lactation and the gestation periods showed the ability of the animal to maintain its mineral supply and reserves on the kind of basal ration fed during the experiments. Considering the quantity of production, the quality and quantity of ration and the type of animal used, the necessity of supplementing the ration with any mineral other than common salt is questionable. Certainly there is no reason apparent from the above data to suspect that the body supply was seriously depleted under the conditions of the experiment.

The increased retention of calcium and phosphorus caused by feeding bone meal may have additional significance other than simply increasing the body reserves in that it may aid in maintaining the milk yield on a higher level for a longer period of time. Meigs (1924) observed that sup-

plementing a timothy hay and grain ration with calcium carbonate delayed a falling off in milk yield. However, this ration did not compare with another containing alfalfa in place of timothy hay plus calcium carbonate for milk production and reproduction. Supplementing legumes with calcium salts and the effect of this procedure on milk yield and reproduction will have to be answered by further investigations.

SUMMARY

1. The mineral balance of the same three animals previously reported was again observed. Two animals were in the gestation period and one again in early lactation.

2. As observed before, kale favorably influenced the milk yield and phosphorus balance. Bone meal also changed the negative calcium and phosphorus balances to positive during heavy lactation.

3. During gestation the two animals stored appreciable quantities of calcium and phosphorus on the basal ration. With the exception of chlorine in one case all the elements showed a positive balance.

4. No depletion of the mineral reserves for the total reproductive cycle could be observed based upon data reported here and previously obtained with a ration of red clover hay, silage and grain.

5. Evidence is presented to show that the alimentary absorption of calcium and phosphorus is controlled to a certain degree by the demands of the animal. The character of the ration also affects the retention of minerals and acid base equilibrium.

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